Role of neural barriers in the pathogenesis and outcome of *Streptococcus pneumoniae* meningitis (Review)

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Abstract. Bacterial meningitis is an inflammatory disease of the meninges of the central nervous system (CNS). *Streptococcus pneumoniae* (*S. pneumoniae*), *Neisseria meningitidis*, and *Haemophilus influenzae* are the major bacterial pathogens causing meningitis with *S. pneumoniae* being responsible for two thirds of meningitis cases in the developed world. To reach the CNS following nasopharyngeal colonization and bacteraemia, the bacteria traverse from the circulation across the blood brain barrier (BBB) and choroid plexus. While the BBB has a protective role in healthy individuals by shielding the CNS from neurotoxic substances circulating in the blood and maintaining the homeostasis within the brain environment, dysfunction of the BBB is associated with the pathophysiology of numerous neurologic disorders, including bacterial meningitis. Inflammatory processes, including release of a broad range of cytokines and free radicals, further increase vascular permeability and contribute to the excessive neural damage observed. Injury to the cerebral microvasculature and loss of blood flow auto-regulation promote increased intracranial pressure and may lead to vascular occlusion. Other common complications commonly associated with meningitis include abnormal neuronal hyper-excitability (e.g., seizures) and loss of hearing. Despite the existence of antibiotic treatment and adjuvant therapy, the relatively high mortality rate and the severe outcomes among survivors of pneumococcal meningitis in developing and developed countries increase the urgency in the requirement of discovering novel biomarkers for the early diagnosis as well as novel treatment approaches. The present review aimed to explore the changes in the brain vascular barriers, which allow *S. pneumoniae* to invade the CNS, and describe the resultant brain injuries following bacterial meningitis.

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Contents

1. Introduction

Streptococcus pneumoniae Meningitis. Despite the implementation of childhood vaccination schemes and the availability of effective antibacterial agents, bacterial meningitis remains to affect individuals in developing and developed countries. Meningitis is a central nervous system (CNS) infection often associated with severe outcomes and significant long-term effects in a substantial number of survivors. This disease thus requires prompt diagnosis and treatment (1). Complications may be sudden or gradual in onset and may arise anytime after the appearance of initial symptoms, including the time after completion of therapy. Symptoms of meningitis include fever, headache, confusion and vomiting, often preceded by symptoms of an upper respiratory tract infection. Clinical features used for diagnosis include cerebrospinal fluid (CSF) pleocytosis with predominance of neutrophils, elevated CSF protein, decreased CSF glucose and isolation of the bacteria from the CSF by culture. *S. pneumoniae*, *Neisseria meningitidis* and *Haemophilus influenzae* are the major bacterial pathogens causing meningitis with *S. pneumoniae* being responsible for two thirds of meningitis cases in the developed world (1).

S. pneumoniae is a Gram-positive pathogen, which colonizes the nasopharyngeal mucosa of children as well as adults and is transferred among individuals by coughing and sneezing (for review, see 2). In addition to meningitis, *S. pneumoniae* causes a broad spectrum of diseases, including otitis media, pneumonia and bacteremia (2), leading to substantial morbidity and mortality worldwide. Mortality due to pneumococcal meningitis ranges between 15 and 40%, while ~50% of survivors experience long-term health effects. Of note, even after a good recovery, neuropsychological testing demonstrates cognitive impairment in approximately one third of survivors (3,4). Vaccination with the pneumococcal conjugate vaccine has reduced pneumococcal invasive diseases caused by the serotypes included in the vaccine, but this reduction has been offset by increases of carriage and disease caused by strains not included in the vaccine formulation (2,5,6). The following review highlights the underlying mechanisms of neural complications following CNS invasion of *S. pneumoniae*.

Colonization. The pneumococcus habitat is the human nasopharynx mucosa with a prevalence of ~40% in infants and 15% in adults (7). The bacterium is transferred between individuals mainly by coughing and sneezing. Once in the niche, bacterial survival depends on adherence, nutrition and replication; the pneumococcus has to overcome the host's immune system as well as other microbial species that can colonize the same niche (8). Two types of pili identified in *S. pneumoniae* are responsible for initial attachment of the bacterium to the host. The first pilus is an oligomeric appendage encoded by the rlrA operon (9) and the second pilus is encoded by pilus islet 2 (10). To reach the epithelial cell layer and colonize the nasopharynx, *S. pneumoniae* degrades the mucus by exoglycosidases such as neuraminidase A, β-galactosidase, β-*N*-acetylglucosaminidase and neuraminidase B, decreasing mucus viscosity and preventing mucus entrapment (11). In addition, it produces pneumolysin, a toxin that decreases epithelial cell cilia beating and enhances bacterial adherence (12). Pneumococcus also expresses the enzymes *N*-acetylglucosamine-deacetylase A and *O*-acetyltransferase that provide resistance to lysozyme that cleaves the peptidoglycan in the cell wall of the pathogen (13). To compete with the host innate immune system at the nasopharynx site, the pneumococcus has developed several strategies: i) Encapsulation and the use of proteases which prevent binding of secretory immunoglobulin (Ig) A and cleave IgA antibodies, immune system components designed to facilitate phagocytosis of the bacteria (14). ii) Inhibition of the activity of Lactoferrin, an iron-binding glycoprotein, thus enabling the utilization of iron, which is necessary for the bacteria's metabolism (15). iii) Limitation of the activation of the complement cascade which normally promotes the cleavage of several complement factors, leading to bacterial opsonization and phagocytosis, leukocyte recruitment and creation of pores in the pathogen's membrane, thus inducing cell lysis (6,16).

Following the initial attachment, adhesive molecules embedded in the bacterial cell wall or the cytoplasmic membrane are exposed as a result of shedding of the capsule (17). Among these adhesins are: The lipoprotein pneumococcal surface adhesin A (18) which binds to the E-cadherin receptor (19); the Pneumococcal adherence and virulence factor (Pav)-A protein, which binds to the extracellular matrix protein fibronectin to produce a Pav-A-finbronectin complex that binds an integrin receptor (20,21); fructose bisphosphate aldolase (22,23), which was found to bind the flamingo cadherin receptor and NADH oxidase, which was found to bind, among others, laminin α5 and contactin 4 (24). Recently, Pav-B and CbpA (CbpA, also known as PspC) were found to have an important role in the interaction of *S. pneumoniae* with matricellular glycoprotein thrombosphondin-1, a mediator of bacterial adhesion to host cells (25).

After binding, *S. pneumoniae* interacts with additional adhesins. These include phosphorylcholine, which binds to the platelet activating factor receptor (PAF-R), CbpA (26) which binds to either the polymeric Ig receptor (pIgR) or to secretory IgA (21). The latter two adhesins are considered invasins, since they facilitate transcytosis through the mucosal epithelial cell layer from the apical membrane to the basal membrane using the PAF-R and pIgR recycling pathways (27). Binding and crossing the epithelial cell layers allows access to the submucosal layer, leading to invasive illness.

Bacteraemia. During the bacteremic phase, the pneumococcus reaches and aims to survive in the bloodstream. In the blood, the pneumococcus confronts additional host defense mechanisms, the first line of defense being complement-mediated opsonization and phagocytosis. To survive, the pneumococcus increases its capsule size, reduces complement deposition on its surface and limits subsequent interaction with phagocytes (28,29). This is achieved by bacterial surface proteins that target specific complement components, thus inhibiting complement-mediated clearance, e.g., by recruiting factor H that inhibits the complement cascade (30,31). The second line of defense is recognition of the bacteria by antigen-presenting cells through the binding of pattern recognition receptors directed specifically toward general motifs of molecules expressed by the pathogen. This results in release of cytokines such as tumor necrosis factor alpha (TNF-α), interleukin (IL)-1 and IL-6 that further induce the recruitment of neutrophils and lymphocytes, and simultaneously cause cerebrovascular damage (32). A prominent risk resulting in vascular injury is exposure of blood to an extravascular tissue factor and activation of the coagulation cascade that may lead to vascular clot formation (33,34), BBB dysfunction and blood flow impairment (35).

2. Pneumococcal invasion of the CNS

A high amount of bacteria in the blood circulation is thought to be required for CNS invasion, and is thus considered as a risk factor for the development of meningitis. Bacteraemia allows the pneumococcus to cross the cerebral endothelium or the choroid plexus epithelium and enter the CNS. Although the blood-CSF barrier and the BBB protect the brain and meningeal space from pathogen attacks, pneumococci have developed mechanisms to overcome this obstacle.

Blood-CNS barriers. The cellular components of the neurovascular unit use a combination of chemical and electrical signals to communicate. Proper neuronal signaling depends on precise ionic concentrations; thus, it is essential to maintain

a stable microenvironment within the neuropil. Three barrier layers limit and regulate molecular exchange at the interfaces between the blood and neural tissue: i) The BBB at the level of cerebrovascular endothelial cells; ii) the blood-CSF barrier at the choroid plexus epithelium in the ventricles; and iii) the arachnoid-membrane barrier, which underlies the dura and envelopes the brain. All three barriers apply a combination of a physical barrier (tight junctions between cells reducing flux through intercellular pathway), a transport barrier (transport mechanisms mediating solute flux) and a metabolic barrier (enzymes metabolizing molecules in transit) (36). The BBB, first described by Goldman (37), exerts the most significant control over the immediate microenvironment of brain cells. It bestows the blood vessels in the CNS with unique properties that tightly regulate the movement of molecules, ions and cells between the blood and the neural tissue. Due to the presence of tight junctions between adjacent endothelial cells which limit para-cellular passage, trafficking across an intact BBB is mostly trans-cellular (through carriers and vesicular systems) (for review see 37-39). An additional route of entry for small lipophilic agents are lipid membranes, through which small gaseous molecules, including oxygen and carbon dioxide, can diffuse freely. Trans-cellular traffic of small hydrophilic molecules is regulated by specific transport systems on the abluminal and luminal membranes to provide a selective 'transport barrier' and prevent the entry of potentially harmful compounds while facilitating or permitting the entry of required nutrients (including glucose, amino acids, nucleosides and monocarboxylic acids) (38). In addition, members of the ATP-binding cassette (ABC) family, with support of metabolizing enzymes (e.g., cytochrome P450 s), act as efflux transporters by reducing the entry into the brain of numerous toxic components from the diet and environment (39). Unless they can be transferred by specific receptor-mediated transcytosis or by the less specific adsorptive-mediated transcytosis, large hydrophilic molecules such as proteins and peptides are generally excluded (40,41). The two main trafficking routes for BBB transcytosis involve clathrin-coated vesicles and caveolae (42,43). Compared to other tissues, the neutrophil infiltration rate into the brain is low due to the strictly regulated immune cell-BBB interaction. In conditions of increased vascular permeability, circulating neutrophils and mononuclear leukocytes, monocytes and macrophages penetrate into the CNS and have a role complementary to microglia, forming cuffs in the perivascular space, which act as a specific niche for a coordinated immune response (44,45). Failure of the BBB appears to be an important, and possibly a critical event in the development and progression of several brain diseases, including stroke, trauma, epilepsy, neurodegenerative diseases, tumors and bacterial infections. Barrier dysfunction can range from slight and transient tight junction opening, malfunction of transcellular transport mechanisms and ABC transporters to severe and long-lasting barrier breakdown (46). In most cases, it is hard to determine whether barrier dysfunction is the direct cause of disease onset, but it has been established that disturbances in barrier functions contribute to and exacerbate developing pathologies.

The choroid plexus, found in all four ventricles, is comprised mainly of a single layer of highly vascularized epithelial cells connected by tight junctions. Macrophages and leukocytes also occupy the choroid plexus, suggesting its role as a gateway for immune cell trafficking in response to disease and trauma. Cells of the choroid plexus produce the CSF, which is secreted across the epithelium into the ventricles, while the interstitial fluid (ISF), which constitutes the remainder of the brain extracellular fluid, is generated at least in part by secretion across the capillary endothelium of the BBB. The secretion of CSF and ISF is regulated by the Na^+, K^+-ATPase, which is expressed in the abluminal side of the BBB endothelium and the apical membrane of the choroid plexus epithelium, and creates the ionic and osmotic gradient that results in flow of water (47,48). The third interface, provided by the arachnoid epithelium, acts as a seal between the extracellular fluids of the CNS and those of the rest of the body (49). Due to its avascular nature and relatively small surface area, the arachnoid does not represent a significant surface for exchange between the blood and the CNS, while it is also regarded as a barrier layer (50).

Pneumococcal trafficking across the barriers. In the pre-antibiotic era, bacterial meningitis was a disease with devastating mortality, which was fatal to virtually all individuals affected (51). However, despite the advent of effective antimicrobial agents, a finite case fatality rate remains, with permanent neurologic sequelae affecting numerous survivors. One potential explanation is that the pathologic consequences of the disease within the CNS progress despite bacterial cure. The pathogen can use two routes to reach the brain, either directly through the endothelium of the BBB or through the choroidal epithelial cells. The BBB is known to be functionally altered in meningitis (52,53), and transit of the bacteria across its endothelium is conceivable via either trans-cellular or para-cellular pathways. Studies investigating the mechanisms of trans-cellular migration showed that the thickness of the bacterial capsule has an important role: While the most opaque variants (thick capsule) were killed, the transparent phase variants (thin capsule) were able to transcytose to the basal surface of the endothelium. This trans-cellular pathway is dependent on the PAF receptor, a choline receptor, and the presence of CbpA that binds the pIgR (54). In addition, the pneumococcal surface protein CbpA enables binding to the laminin receptor on the brain endothelium and subsequent trans-endothelial traffic (55). An alternative mechanism involves inter-cellular migration following disruption of tight junctions: A study using a rat model of meningitis showed that pinocytotic vesicle formation appeared to be an early response to pneumococcal infection in the subarachnoid space. This was followed by a progressive increase in complete separation of intercellular junctions as the infection progressed (56), either due to the pneumococcus itself by the release of compounds such as pneumolysin (57) or as result from inflammatory response (32,58,59). However, certain bacterial infections are thought to start in the choroid plexus prior to becoming diffused, suggesting that this may be the predominant site of abnormality in patients suffering from meningitis (60).

3. Immune response and neuro-inflammatory mechanisms following CNS invasion

For the normal functioning of the brain, protection from blood-borne toxins, proteins and cells is crucial. Accordingly,

a compromised BBB is tightly associated with changes in the extracellular milieu of the brain, activation of glial cells and a neuro-inflammatory response which further contributes to the course of a disease (61). In peripheral organs, the presence and nature of pathogens are detected by innate dendritic cells, which then educate lymphocytes about the specifics of pathogen threat detect through the release of selective mediators, and the lymphocytes are then directed to the site harboring the pathogen (62,63). Compared with inflammatory reactions in other tissues, those within the CNS show substantial differences: i) The CNS parenchyma lacks resident dendritic cells and perivascular macrophages, and the vascular pericytes take over the functions of mature dendritic cells (64); ii) astrocytes, microglia and in certain regions mast cells are the innate parenchymal immune cells of the CNS (65,66); iii) due to the presence of the BBB, extravasation of large molecules and blood cells is reduced. Activation of complement cascades and recruitment of cells involved in the adaptive immune response into the CNS parenchyma is therefore more difficult. Under circumstances of successful CNS invasion, pneumococcus replication occurs concurrently with the release of inflammatory bacterial products (67). Consequently, the brain is infiltrated by blood complement system components, antibodies and neutrophils, resulting in bacterial opsonization and phagocytosis (6).

From pathogen recognition to release of inflammatory molecules. Defense begins with the recognition of pathogen-associated molecular patterns (PAMPs), which are structural signatures characteristic of the bacteria. These include bacterial products such as proteins, lipids, nucleic acids and carbohydrates. PAMPs are initially sensed by antigen-presenting cells which express pattern recognition receptors. The main receptors involved in *S. pneumoniae* recognition are Toll-like 2 receptor (TLR-2, also known as CD282), which are recognized by peptidoglycans and lipoteichoic acids (68), TLR-4 (also known as CD284) that are recognized by exotoxin pneumolysin (69) and TLR-9 (also known as CD289), intracellular pattern recognition receptors that are activated by cytosine-guanosine motifs (CpG) in bacterial DNA (70). TLR-2 and -4 bind to myeloid differentiation factor 88 (MyD88) as a common intracellular adapter protein known, which activates the nuclear factor (NF)-κB pathway with subsequent upregulation of pro-inflammatory mediators. Studies have demonstrated that TLR-2 and -4 have a more prominent role than TLR 9 in the induction of the inflammatory response to pathogens and suggested that one receptor may compensate for the absence of the other (71). In addition, family members of the intracellular nucleotide oligomerization domain (NOD) -like receptors (NLRs), Nod-2, also have essential roles in Gram-positive peptidoglycan detection: When activated, they stimulate NF-κB or mitogen-activated protein kinase pathways and activate caspase-1 (72). This in turn stimulates the production of inflammatory cytokines and chemokines by astrocytes and microglia that express TLRs and NLRs (73). In humans, certain deficiencies and polymorphisms such as phosphorylation of interleukin receptor-associated kinase and Myd88 adapter protein have been associated with invasive pneumococcal disease such as meningitis (74).

4. Immune residents of the brain-microglia and astrocytes

Microglia. As described by Del Rio Hortega, microglia comprise up to 20% of the non-neuronal cell population, are derived from the mesoderm and are considered as the resident macrophages of the CNS (75). The brain of adults has two major subsets of microglia: Parenchymal and perivascular microglia. The latter are located in the basal lamina of brain capillaries and the choroid plexus. The phagocytic properties of microglia (76) have been considered to be the first line of defense in the CNS and to impact numerous immune responses of the brain against infectious and acute as well as chronic neurological diseases (66,77). Resting microglia have small bodies and long, thin processes with ramified morphology, which correspond to the vigilant form which is able to promptly recognize homeostatic disturbances in the CNS (77). Under pathological conditions, microglia become activated and are characterized by an amoeboid morphology with short processes. Surface antigens and cytokine release corresponding to distinct phenotypes are associated with the transition from one form to another (78). The activated, phagocytic phenotype has been indicated to mediate the elimination of neurotoxic substances from brain parenchyma, such as blood-borne albumin (79). Since microglial cells are located in the perivascular space, it is likely that their interactions with endothelial cells influence the properties of the BBB. It has been suggested that, similarly to astrocytes, activation of microglia restores BBB integrity after its chemically stimulated loss by directing tight junction proteins to para-cellular domains (80). However, in neuro-inflammation, activated microglia may cause barrier impairment and BBB dysfunction by releasing the pro-inflammatory cytokine TNF-α (81). Apart from their participation in inflammatory and infectious events and their scavenger function, microglia also take part in several important physiological events in the adult brain, including induction of apoptosis in specific subpopulations of developing neurons, control of synaptogenesis and synaptic transmission as well as synthesis of neurotrophic factors (82).

Astrocytes. Glial astrocytic cells are ideally situated to function as mediators in neurovascular communication; they surround synapses and can thus sense neuronal activity, whereas their end-foot processes envelop blood vessels and may signal (and sense) smooth muscle cells and/or pericytes (83). Astrocytes are enriched in potassium channels, purinergic receptors and the gap junction protein connexin 43, as well as the water-channel protein aquaporin-4 (84), indicating key roles in potassium buffering (85), calcium-dependent glio-vascular signaling and regulation of brain water content. Astrocytes release several vasoactive factors, including nitric oxide (NO) and arachidonic acid metabolites, having a prominent role as mediators of vasomotor activity (86). Moreover, astrocytes produce neutrophins and a wide range of anti-inflammatory cytokines (87). Upon activation of various signaling pathways [e.g. TLR/NLR and transforming growth factor (TGF) beta], astrocytes participate in innate immune reactions, synthesize and release neuro-inflammatory mediators, including several complement components, cytokines such as IL-1β and IL-6, and chemokines (88-92).

Inflammatory mediators. Following recognition of the pathogen and activation of non-neuronal cells, microglia and astrocytes produce and release a wide range of inflammatory molecules in the brain. These include molecules such as cytokines, free radicals, matrix metalloproteinases (MMPs) and chemokines, which contribute to an increase in BBB permeability and enable recruitment of leukocytes to the site of infection. Alongside bacterial elimination, this results with brain edema, increased intracranial pressure and impaired cerebral blood flow, and may lead to irreversible neuronal injury (88).

Cytokines. Cytokines are multifunctional pleiotropic proteins with crucial roles in cellular activation and cell-to-cell communication. Depending on their function, they are classified as being either pro- or anti-inflammatory according to the final balance of their effects on the immune system. Cytokines not only participate in the immune response but also in a variety of physiological and pathological processes, including events in the periphery and CNS; therefore, they act as immune regulators as well as neuromodulators. The neuro-immune interactions are bidirectional-cytokines can modulate the action, differentiation and survival of neurons, while neurotransmitters and neuropeptides released from neurons have a central role in influencing the immune response. The various cytokines directly affecting the CNS either originate from the peripheral immune system, in which case they migrate across the opened BBB, or they are produced locally within the CNS by brain resident cells (89).

Following pneumococcal infection, release of pro-inflammatory cytokines includes molecules such as IL-1, IL-6, TNF-α and interferon (IFN)-γ. These cytokines are released by endothelial cells, astrocytes, microglia and neurons, resulting in increased BBB permeability and recruitment of leukocytes from the circulatory blood (32,90). TNF-α is a 158 amino acid cytokine; it was found to be produced in the cortex and hippocampus during the first 6 h, and remained elevated until 96 h after meningitis initiation (91,92). TNF-α binding to its cognate receptor leads to NF-κB activation that regulates the expression of other pro-inflammatory mediators (93). In patients with bacterial meningitis, intrathecal levels of TNF-α were correlated with the severity of BBB disruption, neurologic sequelae and disease severity (58). However, TNF-α deficient mice infected with *S. pneumoniae* demonstrated increased mortality and spatial memory deficits (94), suggesting that TNF-α reflects disease severity but is not required to induce neurological complications. IL-1β is a pro-inflammatory cytokine produced early after bacterial invasion of the brain in the cortex and hippocampus by perivascular mononuclear phagocytes, macrophages and glial cells through stimulation of bacterial compounds or TNF-α (91,92). IL-1β increases the expression of nearly all other cytokines, including TNF-α, IL-6, IFN-γ and chemokines; however, its role in bacterial meningitis remains elusive. In patients with bacterial meningitis, IL-1β levels were not correlated with the degree of BBB opening (58). By contrast, decreased levels of IL-1β were associated with lower intracranial pressure (ICP), leukocyte recruitment and brain edema (95). Intrathecal administration of IL-1β did not lead to CSF pleocytosis or brain edema, but administration of anti-IL-1β antibodies decreased TNF-α-induced leukocyte influx (96). IL-1 receptor (IL-1R) gene-deficient mice succumbed earlier to the disease and their mortality rate was significantly elevated, indicating that endogenous IL-1β is required for an adequate host defense in pneumococcal meningitis (97). IL-6 is expressed mostly in the cortex and is produced by endothelial cells, astrocytes and monocytes (98). Although the effects of IL-6 are predominantly pro-inflammatory, including leukocyte recruitment, the potent induction of acute-phase proteins and fever (99), it also acts as an anti-inflammatory cytokine. Indeed, IL-6 gene deficiency in mice with bacterial meningitis was found to be associated with an increased inflammatory response and an impaired defense against pneumococcal pneumonia, as well as reduced vascular permeability and ICP (100,101). IFN is another important pro-inflammatory mediator in pneumococcal meningitis, found at elevated concentrations in meningitis patients' CSF (102). It has been suggested that IFN-γ is produced following bacterial recognition through activation of the NLR inflammasome pathways (103,104).

Anti-inflammatory cytokines such as IL-10 and TGF-β have been shown to be upregulated during pneumococcal meningitis. IL-10 is a potent immune-suppressive cytokine produced by brain cells such as neurons and microglia as well as macrophages and monocytes (105), and elevated levels have been found in the CSF of patients with bacterial meningitis (106). IL-10 was shown to inhibit the production of pro-inflammatory cytokines including TNF-α and IL-6, as well as the release of reactive oxygen species (ROS) (107), and to induce impairment of neutrophil phagocytosis and killing (108). Accordingly, systemic administration of recombinant IL-10 in a rat model of pneumococcal meningitis resulted in lower levels of pro-inflammatory cytokines, CSF pleocytosis and cerebral edema (107). However, in IL-10 knockout mice with pneumococcal meningitis, bacterial loads and survival rates were similar to those in wild-type mice (97). TGF-β is a pleiotropic cytokine with potent inflammatory regulatory activity expressed in neurons and glial cells. Among its multiple functions, it modulates T-cell activity, including proliferation and differentiation processes (109,110). The influence of TGF-β on the immune reactivity of the CNS following infection remains to be under debate; it suppresses the production of pro-inflammatory cytokines IL-1β, IL-6 and TNF-α from microglia and macrophages (111,112), but has also been reported to increase the production in cultured astrocytes (113). Absence of TGF-β signaling was demonstrated to facilitate the recruitment of leukocytes and the clearance of *S. pneumoniae* in the CNS of mice with meningitis, resulting in reduced cerebrovascular complications (114).

Chemokines. Chemokines are a subgroup of cytokines, which are considered to have chemotactic activity due to their ability to induce directed chemotaxis in nearby responsive cells. In pneumococcal meningitis, multiple chemokines have been reported to be upregulated and enhance the recruitment and accumulation of inflammatory cells in the CSF (115). IL-8 is produced by a wide range of cells, including macrophages and monocytes, through IL-1β, TNF-α and stimuli of live bacteria. In rabbits with pneumococcal meningitis, intravenous administration of a monoclonal antibody to IL-8 attenuated pleocytosis (116). Another group of chemokines are the CC

chemokine ligands (117), including monocyte chemoattractant proteins and macrophage inflammatory proteins (MIPs), which are released by astrocytes and microglia (118,119). Intracisternal administration of recombinant molecules was found to induce BBB dysfunction, CSF leukocytosis and brain edema (96).

Leukocyte migration. As part of the immune response to bacterial infection, blood-derived leukocytes, such as neutrophilic granulocytes and monocytes, enter the CNS to clear the pathogen. The recruitment of leukocytes through the BBB is associated with meningeal and perivascular macrophage activation and upregulation of the endothelial adhesion molecules selectin and integrin (120,121). The PAF is a protein which facilitates the adhesion of leukocytes to the endothelium and is produced by neutrophils and endothelial cells in response to inflammatory stimulation (122). Another translocation mediator in pneumococcal meningitis is the urokinase-type plasminogen activator, which is also considered as a fibrin degrader (123). However, after migration into the CNS, leukocytes release a variety of toxic molecules (e.g., ROS), causing cerebrovascular complications and neuronal injury (115).

MMPs. MMPs are a family of neutral proteases which are of importance in normal development, wound healing and a large variety of pathological processes, including neuro-inflammation and the spread of metastatic cancer cells (124). In the CNS, MMPs have been shown to degrade components of the basal lamina, leading to BBB breakdown, and contribute to inflammation in numerous neurological diseases. In response to cellular stress, MMPs are secreted by a wide range of cells, including activated neutrophils and macrophages, neurons and glial cells (125). High concentrations of MMP-8 and -9 have been detected in the CSF of patients with bacterial meningitis, and indeed, high concentrations of MMP-9 have been demonstrated to be correlated with TNF-α levels, to induce BBB dysfunction, and to be a risk factor for the development of post-meningitis neurological deficits (125).

Free radicals. A plethora of studies on patients as well as animal models implied that free radicals, including ROS and reactive nitrogen species (RNS), hydrogen peroxide (H_2O_2) and hydroxyl peroxide have a central role in the development of intracranial complications and brain damage in bacterial meningitis (126,127). Brain cells and attracted leukocytes produce free radicals as part of the host immune response to invasive bacterial infection (128,129). *S. pneumoniae* itself is also an important source of H_2O_2, which causes direct cytotoxic damage and also reacts with the host's NO to form peroxynitrite ($ONOO^-$), a highly reactive oxidant (130). $ONOO^-$ is formed at sites where NO and superoxide anions are produced simultaneously (131,132). $ONOO^-$ can be cytotoxic by a number of mechanisms, including tyrosine nitration that affects cellular signaling (133), lipid peroxidation that induces loss of membrane function and integrity (134), and production of cytokines and MMPs (135,136). Adjuvant therapy with an $ONOO^-$ scavenger reduces the number of CSF leukocytes as well as IL-1β and MIP-2 concentrations in the brain (137), which suggests that ROS/RNS and pro-inflammatory chemokines/cytokines are involved in the attraction of blood-bound leukocytes into the subarachnoid space (138). Furthermore, treatment with antioxidants was shown to attenuate BBB leakage (137,139).

5. Brain injury following pneumococcal invasion

In spite of advances in antimicrobial therapies and supportive care, brain injury and mortality associated with and resulting from *S. pneumoniae* infection have remained significant.

Cellular damage. An important histopathological finding in patients with *S. pneumoniae* meningitis as well as in experimental meningitis is cell death in the dentate gyrus of the hippocampus (140), which was also correlated with the development of learning deficits (141,142). *S. pneumoniae* is able to induce two functionally distinct forms of programmed cell death in the brain, which proceed either via TLR-dependent or -independent pathways. The pneumococcal cell wall is a pro-inflammatory component and causes apoptosis in the hippocampus mediated via TLR-2 and activation of caspases (143). Living pneumococci and the major cytotoxins pneumolysin and H_2O_2 appear to induce damage to endothelial cells of the BBB through a TLR-independent pathway (143). The these toxins induce an increase in ROS and intracellular calcium, resulting in mitochondrial dysfunction that leads to the release of apoptosis-inducing factors into the cytosol (130,144).

Cerebrovascular injury. Pneumococcal meningitis is at times accompanied by cerebrovascular complications, including ischemic stroke, venous thrombosis, intracerebral hemorrhage and vasculitis (145-148). Additional studies have suggested that vasospasm, thrombosis and diffused cerebral intravascular coagulation may each contribute to cerebral injury (149-151). Other factors, including alterations in blood pressure and impaired cerebral auto-regulation of blood flow may also have a role (152,153). Following bacterial infection and stroke, cells in the penumbra are subjected to various inflammatory components such as infiltrating leukocytes, pro-inflammatory cytokines and free radicals, which may be deleterious and lead to further cellular damage (154). Furthermore, subcortical ischemic white matter lesions have been identified, which led to axonal injury (155). Recently, the pro-inflammatory cytokine IL-1 was identified as a key mediator of pneumococcal infection-induced cerebral ischemia and neuronal injury (156). In a model of ischemic stroke, bacterial aspiration led to severe pneumonia, which persisted for fourteen days after stroke induction, suggesting that stroke increases the susceptibility to infection (157). Another severe vascular complication in pneumococcal meningitis is venous thrombosis (158,159), associated with impairments of blood flow and perfusion pressure, intracranial hemorrhage, BBB dysfunction and cerebral edema (160,161).

Seizures. Seizures are a common complication of *S. pneumoniae* meningitis during the acute phase of illness, with an increased risk of developing unprovoked seizures (e.g., epilepsy) later in life (162,163). Seizures occur frequently in adult patients and are associated with severe inflammation and structural CNS lesions (164,165). In children, the probability

of the occurrence of status epilepticus associated with fever is higher than that of short febrile seizures, and the classical symptoms and signs of meningitis may be absent under such conditions (166,167).

Studies have suggested that cerebrovascular injury and BBB dysfunction, which allow the infiltration of albumin, lead to activation of TGF-β signaling in astrocytes. This further results in a neuro-inflammatory response associated with up-regulation and release of IL-1β, IL-6 and TGF-β cytokines and seizures (168-172). Thus, in patients with pneumococcal meningitis, increased BBB permeability and inflammatory response as described above may similarly increase the likelihood of seizures.

Labyrinthitis. One of the most common sequelae of pneumococcal meningitis is bacterial labyrinthitis and sensory neural hearing loss. Labyrinthitis occurs as the bacteria reach the cochlear aqueduct from the subarachnoid space or travel with the eighth cranial nerve in the internal auditory canal (173,174), contributing to hair cell injury and neuronal cell death (175). A study using a mouse model of pneumococcal meningitis suggested a pro-inflammatory role for the TLR-MyD88 signaling pathway as a trigger for labyrinthitis (176). Synthesis of TNF-α and free radicals has been demonstrated to have an important role in hearing loss following infection, and inhibition of their production was found to have a protective effect (175,177).

Neuropsychological and mental-status impairment. Mild to severe intellectual and behavioral deficits, including cognitive impairment, learning disabilities and attention deficit hyperactivity disorder are well-recognized complications of bacterial meningitis in children and are the most common long-term sequelae (178,179). Psychiatric illness and neurodegenerative diseases have been linked with cerebrovascular damage and BBB alterations: An elevated CSF/serum albumin ratio in patients suffering from dementia compared to non-demented individuals was found (180), and elevated serum levels of S100 calcium-binding protein B, normally found exclusively in the brain, were demonstrated in patients suffering from depression and schizophrenia (181). In addition, accumulating evidence indicated that immunologic responses have a role in deficits in cognitive function as well as depression; Increases in pro-inflammatory cytokines, including TNF-α, IFN and IL-1β and -6, alongside a relative reduction of the anti-inflammatory cytokine IL-10, were demonstrated in depression. Furthermore, a positive correlation was shown between plasma concentrations of inflammatory mediators, such as IL-1β and IL-6, and the severity of depression symptoms (182,183). These suggest that early vascular injury during meningitis, including BBB dysfunction and neuroinflammatory processes, can be associated with delayed neuropsychological and mental problems.

6. Summary

In spite of the wealth of data accumulated on pneumococcal meningitis, as well as the existence of antibiotics, vaccination protocols and adjuvant treatments (e.g., drugs targeting free radicals, the caspase cascade and inflammatory mediators), CNS invasion by *S. pneumonia* results in severe neuropathologies. Several studies point to a direct contribution of BBB dysfunction and inflammatory signaling to the etiology of brain diseases, including those caused by bacterial infection. Thus, following diagnosis of peripheral infection with *S. pneumonia*, imaging of cerebrovascular permeability and measurement of neuro-inflammatory mediators in blood and CSF may aid in identifying patients that are at higher risk of contracting meningitis. Furthermore, since bacterial infection of the CNS requires crossing through the dysfunctional endothelium of BBB and the epithelium of the choroid plexus, cerebral vessels may serve as a potential target for preventing and treating bacterial meningitis.

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