Abstract

Invasion of the central nervous system (CNS) by microorganisms is a severe and frequently fatal event during the course of many infectious diseases. It may lead to deafness, blindness, cerebral palsy, hydrocephalus, cognitive impairment or permanent neurological dysfunction in survivors. Pathogens can cross the blood-brain barrier by transcellular migration, paracellular migration and in infected macrophages. Pathogens may breach the blood-brain barrier and be recognized by antigen-presenting cells through the binding of Toll-like receptors. This induces the activation of nuclear factor kappa B or mitogen-activated protein kinase pathways and subsequently induces leukocyte infiltration and proliferation and the expression of numerous proteins involved in inflammation and the immune response. Many brain cells can produce cytokines, chemokines and other pro-inflammatory molecules in response to bacteria stimuli; as a consequence, polymorphonuclear cells are attracted and activated, and release large amounts of superoxide anion and nitric oxide, leading to peroxynitrite formation and oxidative stress. This cascade leads to lipid peroxidation, mitochondrial damage and blood-brain barrier breakdown, contributing to cellular injury during neuronal infection. Current evidence suggests that bacterial CNS infections can play a role in the etiopathogenesis of behavioral disorders by increasing pro-inflammatory cytokines and bacterial virulence factors. The aim of this review is to summarize the current knowledge of the relevant pathophysiologic steps in CNS infections.

© 2013 Associação Brasileira de Psiquiatria. Published by Elsevier Editora Ltda. All rights reserved.
Introduction

Bacterial infection of the central nervous system (CNS) is a life-threatening condition with a high rate of mortality. Even with protective barriers, pathogens can reach the CNS. The replication of these microorganisms within the CNS occurs in association with the release of compounds such as peptidoglycans, cell wall fragments, lipoteichoic acids (from Gram positive bacteria), lipopolysaccharides (from Gram negative bacteria) and exotoxins, which are highly immunogenic and may elicit an increased inflammatory response in the host. The host’s inflammatory response against the infection has been increasingly recognized as playing a central role in neurological morbidity and mortality. CNS infections may present clinically as meningitis, meningoencephalitis and, more rarely, myelitis.

Cognitive outcomes after bacterial meningitis include impairment of memory, decreased psychomotor performance and impairment of attention/executive functions. In neonatal meningitis, the main pathogens are Streptococcus agalactiae and Escherichia coli K1, which together are responsible for at least two thirds of all meningitis deaths in Europe. Listeria monocytogenes, also causes meningitis, usually in immunocompromised individuals and in pregnant women. E. coli K1 infection includes cognitive involvement in 50% of patients. The main bacterial agents causing meningitis in young children and adults are Streptococcus pneumoniae and Neisseria meningitidis, with the former being responsible for two-thirds of cases in Europe and the United States. Pneumococcal meningitis leads to cognitive impairment in 30 to 52% of surviving patients, including learning difficulties, cognitive slowness, short-term memory deficits and poor academic performance. The aim of this review is to summarize the current knowledge of the relevant pathophysiologic steps of CNS infections: (a) bacterial crossing through the blood-brain barrier; (b) bacterial survival and growth within the CNS; (c) bacterial induction of CNS inflammation; and (d) the role of bacterial infection in the development of behavioral disorders.

Microbial traversal of the blood-brain barrier

The CNS is protected by a bony skull, the leptomeninges, the blood-brain barrier (BBB) and the blood-cerebrospinal fluid barrier. The BBB is composed of brain microvascular endothelial cells, astrocytes and pericytes. It maintains the neural microenvironment by regulating the flux of molecules into and out of the brain, and protects the brain from microorganisms and toxins that come from the blood. Pathogens can cross the BBB by transcellular migration, paracellular migration and by “hitch-hiking” inside infected macrophages. Using transcellular migration, pathogens can cross the BBB without any evidence of intercellular tight-junction disruption or detection of microorganisms between endothelial cells. S. agalactiae, S. pneumoniae, E. coli and N. meningitidis reach the CNS through this mechanism (Figure 1a). The paracellular traversal mechanism involves the penetration of the pathogen between barrier cells, with or without evidence of tight-junction disruption. Borrelia sp and Treponema pallidum cross the BBB through this mechanism (Figure 1b). Other pathogens can cross the BBB inside infected macrophages. L. monocytogenes enters host cells during internalization of phagosomal vacuoles, and produces phospholipase C and the pore-forming cytolsin listeriolysin O to escape the phagosome and replicate within the host cytosol. Mycobacterium tuberculosis also crosses the BBB while residing in the phagosomes of macrophages (Figure 1c).

Mechanisms of innate immunity in the CNS

The innate immune response represents the first line of defense against invading microorganisms. Bacterial compounds such as peptidoglycans, cell wall fragments, lipopolysaccharides and lipoteichoic acid, collectively known

Figure 1 Mechanisms of microbial traversal of the blood-brain barrier: a. Transcellular traversal b. Paracellular traversal and c. the “Trojan horse” mechanism.
as pathogen-associated molecular patterns (PAMPs) are highly immunogenic and elicit strong inflammatory responses in the host. These compounds are recognized by antigen-presenting cells through binding to pattern recognition receptors (PRRs), including the Toll-like receptors (TLRs). Eleven members of the TLR family have been described to date in humans. TLRs are either expressed at the cell surface for extracellular ligand recognition or localized to endosomal compartments for the recognition of pathogen-associated nucleic acids. Microglial cells express all TLRs identified to date, astrocytes express TLR 2, 3 and 9, neurons express TLR 3, 7, 8 and 9 and oligodendrocytes express TLR 2 and 3. Most TLRs transduce their signal through a common intracellular adapter protein known as myeloid differentiation factor 88 (MyD88). MyD88 is associated with Interleukin-1 (IL-1) and receptor-associated kinase-4 (IRAK-4), which is a serine/threonine kinase that plays an essential role in signal transduction by Toll/IL-1 receptors (TIRs). Subsequently, IRAK interacts with the tumor necrosis factor (TNF) receptor-associated factor (TRAF) family, providing a link to NF-κB-inducing kinase, resulting in the nuclear translocation of NF-κB. NF-κB comprises a closely associated family of transcription factors, which play a key role in the expression of genes implicated in the development of accessory cell and lymphocyte populations. NF-κB is also a transcriptional activator of various genes involved in neuronal pathogenesis and in the production of cytokines and chemokines. IL-1β production requires a second signal that is provided by intracellular protein complexes known as inflammasomes. The NLRP3 (cryopyrin) and the AIM2 (absent in melanoma 2) inflammasomes, which are activated by the exotoxin pneumolysin and bacterial DNA in pneumococcal infection, respectively, mediate cleavage of pro-IL-1β into mature IL-1β. NLRP3 has been implicated in responses to the following bacteria: Staphylococcus aureus, L. monocytogenes, Klebsiella pneumoniae and E. coli. Thus, when bacteria reach the CNS, PAMPs are recognized by PRRs, initiating the activation of the host immune response, which triggers the production of pro-inflammatory cytokines and chemokines and the expression of co-stimulatory molecules (Figure 2). In response to these stimuli, leukocytes are attracted from the blood and activated. For instance, polymorphonuclear leukocytes cross the BBB by binding to selectins E and P along with IL-8 (CXCL-8). TNF-α induces production of adhesion molecules ICAM-1 and ICAM-2, which allow extravasation of the leukocyte along chemoattractant concentration gradients. Leukocytes work to eliminate the invading pathogen through a rapid and robust production of reactive oxygen species (ROS). They release high amounts of superoxide anion (O₂⁻) and nitric oxide (NO), generating peroxynitrite (ONOO⁻). The resulting oxidative stress leads to activation of cytokines, enhancing leukocyte activation, lipid peroxidation, mitochondrial damage and metalloproteinase activation. In humans with meningococcal meningitis, TNF-α levels in the serum are correlated with

![Figure 2 Pathogenesis and pathophysiology of bacterial CNS infections.](image-url)
fatal outcomes. TNF-α is released into serum before IL-6 in meningococcal septic shock. High serum levels of IL-6 are also associated with unfavorable outcomes. In addition, IL-1 was exclusively detected in patients who also had high serum levels of IL-6, TNF-α, LPS and rapid fatal outcomes. However, in the CSF of patients with meningitis, higher concentrations of TNF-α were observed than patients with septic shock/bacteremia and the CSF/blood glucose was inversely correlated with TNF-α, IL-6 and IL-1. Patients with pneumococcal meningitis also exhibit a cerebral production of TNF-α, IL-1β and IL-6.

Neuronal damage in the context of bacterial infection of the CNS

Brain injury and neuronal death are not mediated simply by the presence of a viable pathogen, but they also occur as a consequence of the host’s reaction to bacterial compounds. Bacterial cell wall compounds, mainly polysaccharide capsules, are most likely the most important virulence factor. Bacterial cell wall compounds trigger a host inflammatory response from leukocytes and activate the tumor suppressor protein p53 and the ataxia telangiectasia-mutated (ATM) kinase, triggering caspase dependent-apoptosis. The presence of the capsule also prevents the binding of iC3b (a complement factor that stimulates phagocytosis) and Fc (which stimulates receptor-mediated phagocytosis) to the bacterial cell surface, affecting phagocytic activity. Pathogens such as S. pneumoniae, N. meningitidis, E. coli K1 and H. influenzae use this pathogenic mechanism. The virulence of other bacteria is based on the production of enzymes such as coagulase, proteolytic enzymes, hyaluronidase, neuraminidase and catalase. Hemolysin and cytolyticin from Streptococcus have the ability to cause inflammatory activation, and these same enzymes produced by S. agalactiae induce chemokines and ICAM-1 in brain microvascular endothelium cells. Teichoic acids present in Gram+ bacteria can induce TNF-α and NO production, as well as the expression of ICAM-1. Exotoxins are also produced by some bacteria as part of their growth and metabolism, while endotoxins are part of the external portion of Gram (-) bacteria. S. pneumoniae bacterial compounds like peptidoglycans and lipoteichoic acids are recognized in the CNS by TLR2. The exotoxin pneumolysin is recognized by TLR4 and bacterial DNA is recognized by TLR9, which is an intracellular PRP activated by CpG. Pneumolysin and H2O2 are produced and released by pneumococcus, causing neuronal cell death through mitochondrial damage. This damage leads to the release of apoptosis-inducing factor (AIF) into the cytosol. AIF induces apoptosis by a caspase-independent pathway. When pneumococcal cell wall compounds are released, they activate the host immune response and a large number of polymorphonuclear cells are attracted. p53 and ATM converge on mitochondria to initiate the release of cytochrome-c, which is necessary to form the apoptosome (Apafl-1) and activate caspase-9, resulting in activation of caspase-3 and caspase-dependent apoptosis. S. agalactiae produces lipoteichoic acid that is through to activate TLR2, while TLR7 and/or TLR8 interact with microbial RNA. In vitro, L. monocytogenes cell wall components such as lipoteichoic acids are recognized by TLR2 with the help of CD14 and TLR6. The protein flagellin is recognized by TLR5. L. monocytogenes is a classic example of a cytosol-adapted pathogen. It can escape quickly from the phagosomes of macrophages (or other cell types) and it reproduces rapidly in the cytosol. This microorganism also expresses the toxin listeriolysin O, which is responsible for forming pores in the cell and causing lymphocyte apoptosis. E. coli produces the endotoxin lipopolysaccharide, which is recognized by TLR4. Furthermore, E. coli damages the microvascular endothelial cells that constitute the BBB in the human brain. Another important pathogen, S. pyogenes, produces bacterial virulence factors such as, M protein, hyaluronic acid, capsule, fibronectin-binding proteins, streptolysin O and streptolysin S. The M protein binds to complement factors and other host proteins, allowing the pathogen to avoid activation of the alternate complement pathway evade phagocytosis. The M protein has also been implicated in the internalization process, which involves cytoskeletal rearrangements. It is possible that these proteins provide an intrapithelial refuge for the organism, sheltered from phagocytes and humoral antibodies. Another intracellular bacterium, Mycobacterium tuberculosis, infects the CNS and causes different manifestations of disease, like meningitis, space-occupying lesions in the brain parenchyma, and focal disease of the spinal cord and its osseous structures. In previous studies of S. agalactiae meningitis, oxidative damage was elevated and enzymatic defense was decreased. TNF-α, IL-1β, IL-6 and cytokine-induced neutrophil chemoattractant-1 (CINC-1) levels were increased in the first hours after neonatal meningitis induction, prior to BBB breakdown in the hippocampus and cortex. In infant rats with pneumococcal meningitis, the peak of pro-inflammatory cytokine and chemokine production was also associated with a disruption of the BBB. TNF-α and CINC-1 levels were increased in the blood from jugular veins in comparison with arterial blood in the first hours after pneumococcal meningitis induction, indicating their production in the CNS. Moreover, animals that survived bacterial meningitis showed impairments of memory and learning, depressive-like-behavior and anxiety-like symptoms.

Behavioral disorders

Neuronal infection causes intense an inflammatory host immune response; it is potentially fatal and contributes to neurological symptoms. There are several studies describing a probable role of cytokines and oxidative stress in behavioral disorders. Epidemiological research over the last twenty years has indicated that the risk for schizophrenia is enhanced in offspring exposed to viral or bacterial infections in utero, possibly by a disruption in the programmed maturation of the brain in prenatal and early neonatal life. This association between prenatal exposure to bacterial infection and risk of schizophrenia was evaluated, for example, in the Copenhagen Perinatal Cohort, which showed that prenatal exposure to bacterial infections in pregnant women were correlated with an increased risk of schizophrenia in their offspring. It has been proposed that this association might be mediated through transplacental passage of maternally produced cytokines in response to these bacterial infections. Individuals with meningitis during childhood had a 4.4-fold increased risk for schizophrenia and a 4.6-fold
increased risk for psychosis in adulthood when compared to their siblings without childhood meningitis. The immune system may signal the CNS through the action of cytokines, which have also been implicated in the development of schizophrenia. For instance, IL-18 is capable of inducing the differentiation of mesencephalic progenitor cells into dopaminergic neurons. IL-6 is highly effective in decreasing the survival of fetal brain serotonergic neurons and is increased in patients with schizophrenia.

There was a significant association between maternal IL-8 levels during the second trimester of pregnancy and the risk of schizophrenia in the offspring. Cytokines in the CNS are also implicated to depressive disorders. Animals subjected to pneumococcal meningitis and sepsis models had increased levels of pro-inflammatory cytokines in parallel with depressive-like behavior. Interestingly, treatment with imipramine reversed these depressive-like symptoms and decreased TNF-α levels in the cortex 10 days after pneumococcal meningitis induction. Impairment of learning and memory has also been demonstrated in bacterial meningitis in a neonatal animal model. Neonatal Wistar rats subjected to S. agalactiae meningitis showed aversive memory impairment in adulthood and a decrease of the brain-derived neurotrophic factor (BDNF) levels in the hippocampus and cortex. Furthermore, the decreased BDNF levels in the hippocampus were correlated with memory impairment in adult animals subjected to pneumococcal meningitis in the neonatal period. Other neuronal disorders are also related to bacterial infection. Obsessive-compulsive disorder or “tic” disorders are examples of pediatric neuropsychiatric disorders that have been associated with autoimmune disease secondary to S. pyogenes, a group A B-hemolytic streptococcal infection. The involvement of the host immune response is suggested by quantitative changes in circulating levels of TNF-α, IL-18 and IL-6 levels in these patients. Pathogenic mechanisms include antibodies targeting the streptococcal epitope GlcNAc cross-reacting with neuronal molecules like tubulin, lysoganglioside and dopamine receptors. Cytokines have an important role in the pathophysiology of brain infections, and they are implicated in a variety of common diseases that have been associated with production of cytokines, such as psychiatric disorders.

**Conclusion**

Understanding the interactions between the complex networks of cytokines, other inflammatory mediators and leukocytes, and bacterial virulence factors may help to establish more effective therapeutic strategies for CNS infections and, therefore, a better outcome for affected subjects. Moreover, these diseases highlight the role of the immune system in the pathophysiology of psychiatric disorders, which represents an interesting research venue.

**Acknowledgments**

This research was supported by grants from CNPq, FAPESC, FAPEMIG, UNESC, NENASC project (PRONEX program CNPq/FAPESC), INCT-TM and L’Oreal-UNESCO Brazil Fellowship for Women in Science 2011.

**Disclosures**

Tatiana Barichello

**Employment:** Laboratório de Microbiologia Experimental e Instituto Nacional de Ciência e Tecnologia Translacional em Medicina, Programa de Pós-Graduação em Ciências da Saúde, Universidade do Extremo Sul Catarinense, Criciúma, SC, Brazil.

**Jaqueline S. Generoso**

**Employment:** Laboratório de Microbiologia Experimental e Instituto Nacional de Ciência e Tecnologia Translacional em Medicina, Programa de Pós-Graduação em Ciências da Saúde, Universidade do Extremo Sul Catarinense, Criciúma, SC, Brazil.

**Graziele Miloloi**

**Employment:** Laboratório de Microbiologia Experimental e Instituto Nacional de Ciência e Tecnologia Translacional em Medicina, Programa de Pós-Graduação em Ciências da Saúde, Universidade do Extremo Sul Catarinense, Criciúma, SC, Brazil.

**Samuel G. Elias**

**Employment:** Laboratório de Microbiologia Experimental e Instituto Nacional de Ciência e Tecnologia Translacional em Medicina, Programa de Pós-Graduação em Ciências da Saúde, Universidade do Extremo Sul Catarinense, Criciúma, SC, Brazil.

**António Lúcio Teixeira**

**Employment:** Laboratório Interdisciplinar de Investigação Médica, Faculdade de Medicina, Universidade Federal de Minas Gerais, Belo Horizonte, MG, Brazil.

* Modest

** Significant

***Significant. Amounts given to the author’s institution or to a colleague for research in which the author has participation, not directly to the author.

**References**


Neisseria meningitidis


