Pathophysiology of acute meningitis caused by *Streptococcus pneumoniae* and adjunctive therapy approaches

Fisiopatologia da meningite ocasionada pelo *Streptococcus pneumoniae* e novas possibilidades terapêuticas adjuvantes

Tatiana Barichello[1], Jaqueline S. Generoso[1], Allan Collodel[1], Ana Paula Moreira[1], Sérgio Monteiro de Almeida[2]

ABSTRACT

Pneumococcal meningitis is a life-threatening disease characterized by an acute purulent infection affecting piamater, arachnoid and the subarachnoid space. The intense inflammatory host's response is potentially fatal and contributes to the neurological sequelae. *Streptococcus pneumoniae* colonizes the nasopharynx, followed by bacteremia, microbial invasion and blood-brain barrier traversal. *S. pneumoniae* is recognized by antigen-presenting cells through the binding of Toll-like receptors inducing the activation of factor nuclear kappa B or mitogen-activated protein kinase pathways and subsequent up-regulation of lymphocyte populations and expression of numerous proteins involved in inflammation and immune response. Many brain cells can produce cytokines, chemokines and others pro-inflammatory molecules in response to bacteria stimuli, as consequence, polymorphonuclear are attracted, activated and released in large amounts of superoxide anion and nitric oxide, leading to the peroxynitrite formation, generating oxidative stress. This cascade leads to lipid peroxidation, mitochondrial damage, blood-brain barrier breakdown contributing to cell injury during pneumococcal meningitis.

Key words: *Streptococcus pneumoniae*, meningitis, cytokines, chemokines, oxidative stress.

RESUMO

A meningite pneumocócica é doença potencialmente fatal caracterizada por infecção aguda purulenta que afeta a pia-máter, a aracnoide e o espaço subaracnoide. A resposta inflamatória do hospedeiro é potencialmente fatal e contribui para as sequelas neurológicas. O processo inicia-se com a colonização da nasofaringe pelo *Streptococcus pneumoniae*, seguida de invasão, bacteremia e passagem através da barreira hematoencefálica. O *S. pneumoniae* é reconhecido por células apresentadoras de antígenos através da ligação aos receptores Toll-like. Isto induz a ativação do fator nuclear kappa B ou proteína quinase ativada por mitógenos. Muitas células cerebrais também podem produzir citocinas, quimiocinas e outras moléculas pró-inflamatórias em resposta aos estímulos bacterianos. Como consequência, são atraídos polimorfonucleares, ocorrendo a liberação de grandes quantidades de ânion superóxido e óxido nítrico, o que leva à formação de peroxinitrito e ocasiona o estresse oxidativo. Esta cascata pró-inflamatória leva à peroxidação lipídica, a danos mitocondriais e à ruptura da barreira hematoencefálica, contribuindo para o dano celular em meningite pneumocócica.

Palavras-Chave: *Streptococcus pneumoniae*, meningite, citocinas, quimiocinas, estresse oxidativo.

Bacterial meningitis is the most common and serious bacterial infection of the central nervous system (CNS), characterized by an acute purulent infection of the piamater, arachnoid and subarachnoid space[1]. Approximately 1.2 million cases are estimated to occur annually world-wide, resulting in 135,000 deaths[2,3]. ***Streptococcus pneumoniae*** and ***Neisseria meningitidis*** are the main etiologic agents responsible for most of the meningitis cases in Europe and in the USA with up to 61% of total cases[1]. In Brazil, in 2011, among all age groups according to the ***Sistema de Informação de Agravos de Notificação*** (SINAN) were confirmed 8,676 meningitis cases, from these 37% (n=3,194) were bacterial meningitis and 41% (n=3,562) were viral meningitis. Among all the bacterial meningitis cases 35% (n=1,133) were meningococcal; 43% (n=1,383) were meningitis by other bacteria and 15% (n=487) by pneumococcus[4].

[1]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.

[2]Laboratório de Virologia, Hospital de Clínicas, Universidade Federal do Paraná e Instituto de Pesquisa Pelé Pequeno Príncipe, Curitiba PR, Brazil.

Correspondence: Tatiana Barichello; Laboratório de Microbiologia Experimental – Universidade do Extremo Sul Catarinense; Avenida Universitária, 1.105; 88806-000 Criciúma SC, Brasil; E-mail: tba@unesc.net

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Pneumococcus is the most severe cause of bacterial meningitis resulting in a 20-30% of hospital mortality an up to a 40% rate of intracranial complications such as brain edema, hydrocephalus and intracranial hemorrhage[1,5]. Even patients with good apparent recovery may present sequelae; moreover, around one-third of the patients have cognitive impairments[5]. These impairments consist mainly of cognitive slowness, learning impairment, deafness, blindness, neuropsychiatric impairments, cerebral palsy, seizure disorders and mental retardation[6]. The objective of this paper was to review some aspects of the meningitis pathophysiology caused by *S. pneumoniae* and discuss some possible therapeutic adjunctive approaches.

MECHANISMS OF BACTERIAL COLONIZATION

The pneumococcus habitat is the human nasopharynx mucosa with a prevalence of about 40% in infants and 15% in adults[7]. The bacterium is transferred among people by coughing and sneezing. It has to face the natural barrier, the host's immune system and up to 700 different microbial species that can colonize the same niche[8,9]. *S. pneumoniae* colonizes the nasopharynx by degradation of the mucus by exoglycosidases such as neuraminidase A, β-galactosidase, β-N-acetylglucosaminidase, and neuraminidase B decreasing mucus viscosity[10]. *S. pneumoniae* produces the pneumolysin that is a major exotoxin. It decreases epithelial cell ciliary's beating and enhances bacterial adherence[11]. Pneumococcus also expresses the enzymes peptidoglycan, N-acetylglucosamine-deacetylase A, and O-acetyltransferase that provides resistance to lysozyme[12] and it also produces IgA1 protease, which cleaves IgA, the major class of Ig in secretions, promoting binding to the respiratory mucosa[13]. *S. pneumoniae* may transmigrate through the epithelial cells by binding the phosphorylcholine with the receptor of the platelet-activating factor (PAF) or by connecting the pneumococcal choline-binding protein with the epithelial polymeric immunoglobulin receptor, which transports the bacterium to the basal membrane of the host's epithelial cell and may lead to invasive illness[8,14].

CENTRAL NERVOUS SYSTEM BACTERIAL INVASION

The CNS protection is formed by bony skull, the leptomeninges, the blood-brain barrier (BBB) and blood-cerebrospinal fluid barrier[15]. The BBB is constituted of brain microvascular endothelial cells, astrocytes, and pericytes. It maintains the neural microenvironment by regulating the passage of molecules into and out of the brain, and preserves the brain from whatever microorganisms and toxins that comes from the blood[16]. The *S. pneumoniae* crosses the BBB and interacts with cell-wall phosphorylcholine, the platelet-activating-factor receptor and cross the BBB without any evidence of intercellular tight-junction disruption or detection of microorganism between cells through transcellular traversal mechanism[16]. It can also crosses intercellularly by disruption of the interepithelial tight junctions[8]. The pneumococcus replication within the subarachnoid space occur concurrently with the release of the bacterial products such as peptidoglycan, cell wall fragments that are highly immunogenic and may lead to an increased inflammatory response in the host[15]. *S. pneumoniae* is recognized by antigen-presenting cells binding to a pattern recognition receptors. The main pattern recognition receptors involved in initial pneumococcus sensing in the CNS are Toll-like 2 receptors (TRL-2 or CD282) that is recognized by peptidoglycans and lipoteichoic acids[17], Toll-like 4 receptors (TRL-4 or CD284) that are recognized by exotoxin pneumolysin[18] and the Toll-Like 9 receptors (TRL-9 or CD289), an intracellular pattern recognition receptor that is activated by CpG in bacterial DNA[19]. Family members of the NOD-like receptors (NLRs), which are intracellular, play essential roles on innate immunity by detecting intracellular pathogen-associated molecular patterns[20]. When they are activated, they induce the activation of nuclear factor kappa B (NF-κB) or mitogen-activated protein kinase (MAPK) pathways and inflammatory caspases[21]. TRL-2 and TRL-4 use a common intracellular adapter protein known as myeloid differentiation factor 88 (MyD88)[8]. MyD88 signals for NF-κB activation and subsequent up-regulation of pro-inflammatory mediators. MyD88-deficient mice displayed a markedly diminished inflammatory host response in the CNS, as an evidenced of the reduced CSF pleocytosis and expression of cytokines, chemokines and complement factors[22]. Furthermore, MyD88-dependent immune response contributes to hearing loss in experimental pneumococcal meningitis; it is required for mounting a robust host immune response to *S. pneumoniae* in the CNS[23]. NF-κB comprises a closely related family to transcription factors, which play a key role on the expression of genes involved in the development of accessory cell and lymphocyte populations, expressing numerous proteins involved in inflammation and immune response[24]. It is also a transcriptional activator of many genes involved in the pathogenesis of pneumococcal meningitis, such as, TNF-α, IL-1β, inducible nitric oxide synthase and intercellular adhesion molecules[25,26] (Figure).

INFLAMMATION

Cytokines

Many brain cells such as astrocytes, glial cells, endothelial cells, ependymal cells, and resident macrophages can produce cytokines and pro-inflammatory molecules in response to bacterial replication and its components[27]. TNF-α is a 158 amino acid cytokine considered a pro-inflammatory molecule, enhancing the immune response to help speed-up the

pathogens elimination and the resolution of the inflammatory challenge[28]. TNF-α leads to NF-κB activation in the CSF and brain resident cells, which regulates the expression of many pro-inflammatory mediators[29]. In animal models for pneumococcal meningitis, TNF- α was produced in the first 6 h of the immune response[30]. Intrathecal administration of TNF-α results in a similar pathophysiological characteristic of bacterial meningitis such as BBB disruption, facilitating bacterial traversal into the CSF[31], on the other hand, TNF-α deficient mice increased mortality and spatial memory deficits[32]. TNF-α is a marker of the acute inflammatory response, however, it's also essential for an adequate host immune response[8].

IL-1β is a pro-inflammatory cytokine, produced by perivascular, mononuclear phagocytes, glial cells, and meningeal macrophages that increase the expression of nearly all other cytokines such as TNF-α, IL-6, IFN-γ, and chemokines. IL-1β has potent stimulatory effects on granulocytes white cells; it promotes the adhesion of neutrophils and monocytes in endothelial cells[33]. IL-1β is found in the CSF of patients with bacterial meningitis[34], furthermore, in animal models it was produced in the first 24 h after pneumococcal meningitis induction[30], although intrathecal administration of IL-1β did not lead to CSF pleocytosis or brain edema[35]. However, the mortality was significantly higher and earlier in the course of the disease among IL-1 receptor (IL-1R) gene-deficient mice, demonstrating that endogenous IL-1β is required for an adequate host defense in pneumococcal meningitis[36].

IL-6 is produced by monocytes, endothelial cells, and astrocytes primarily in response to IL-1β[37]. It has predominantly pro-inflammatory effects such as potent inducer of acute-phase proteins, fever and leukocytes[38] but it also acts as an anti-inflammatory cytokine, indeed, the lack of IL-6 enhances inflammatory response but decreases vascular permeability in bacterial meningitis[39]. IL-6 gene-deficient mice showed impaired defense against pneumococcal pneumonia[40].

IL-10 is a potent immunosuppressive cytokine, produced by monocytes, macrophages, B and T lymphocytes, brain cells such as neurons and microglia[41]. Elevated levels have been found in the CSF in patients with bacterial meningitis[42], it leads to macrophage and monocyte deactivation and inhibits the cytokines production such as TNF-α and IL-6 and the release of reactive oxygen species[43]. IL-10 gene-deficient mice were associated with higher levels of TNF-α and IL-6 in animal model of pneumococcal meningitis[36].

TGF-β is an anti-inflammatory cytokine that is expressed in neurons and glial cells, maintains T cell tolerance to self or innocuous environmental antigens via its direct effects on the differentiation, homeostasis and regulatory T cells[44]. It suppresses the production of IL-1β, IL-6 and TNF-α from microglia *in vitro*[45], moreover, endogenous TGF-β suppresses the host defense in the CSF of mice with ***S. pneumoniae*** meningitis[3]. The collective activity of TGF-β and IL-10 ensures a controlled inflammatory response specifically targeting

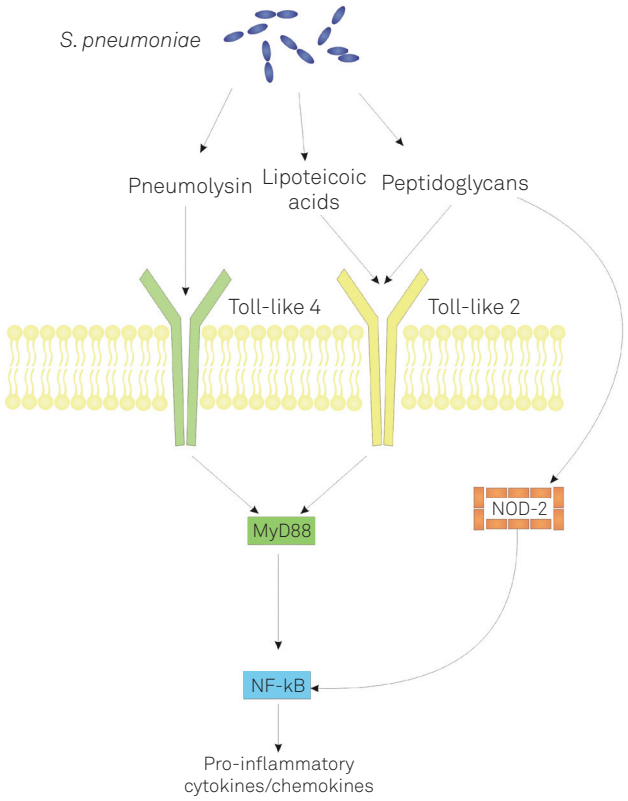

Figure. Summary of the development of host pattern recognition receptors involved in sensing *S. pneumoniae*. Toll-like 2 is activated by pneumococcal cell wall peptidoglycan, lipoteichoic acids and Toll-like-4 is activated by pneumolysin, both use a common intracellular adapter protein MyD88 to activated NF-κB. NOD-2 is activated by peptidoglycan and also activated NF-κB, inducing transcription of several pro-inflammatory mediators.

pathogens without evoking excessive immunopathology to the self-tissues[44].

Chemokines are chemoattractant cytokines, which play key roles on the accumulation of inflammatory cells in the inflammation site. Increased chemokines levels have been reported in the CSF of patients with bacterial meningitis. IL-8 is produced by wide variety cell types such as monocytes, macrophages through stimuli of the live bacteria, TNF-α, and IL-β. It is important to regulate the acute inflammatory response and it's rapidly synthesized in the inflammation sites with the function to recruit acute inflammatory cells[46]. In pneumococcal meningitis treatment with a monoclonal antibody to IL-8 given intravenously attenuated the pleocytosis in rabbits. IL-8 plays an important role on the recruitment of neutrophils during experimental pneumococcal meningitis[47].

Matrix metalloproteinases

Matrix metalloproteinases (MMPs) is a family Zn^{2+} and Ca^{2+} dependent endopeptidases, which is secreted by all cell types in the CNS and serve as effectors of cell migration, tissue remodeling, degrade constituents of the BBB

and interacts with the cytokines[48]. Gelatinases (MMP-2 and MMP-9) have shown to induce BBB breakdown and facilitate leukocyte extravasation in experimental bacterial meningitis[49], furthermore, patients with bacterial meningitis present high concentrations of MMP-939 and MMP-8 in the CSF, indeed, high concentrations of MMP-9 were correlated with risk factor for the development of postmeningitis neurological sequelae[48]. In a rat model, adjuvant treatment with dexamethasone resulted in a lower MMP-9 mRNA[50], in addition, combining the MMP and TNF-α inhibitors led to a decrease incidence of the seizures and mortality, furthermore, neuronal necrosis in the cortex and apoptosis in the hippocampus were attenuated in rats submitted by pneumococcal meningitis[51].

Oxidative stress

Bacterial components are recognized by Toll-like receptors or other pathogen recognition receptors that lead to the activation of NF-κB[21]. It triggers the expression of pro-inflammatory cytokines, as consequence, polymorphonuclear are attracted, activated and released in large amounts superoxide anion (O_2^-) and nitric oxide (NO), leading to the peroxynitrite formation ($ONOO^-$)[52]. The release of large amounts of reactive nitrogen species (RNS) and reactive oxygen species (ROS) had been documented in patient's populations, likewise, in animal model by pneumococcal meningitis and might contribute to the development of neuronal damage[52]. Treatment with superoxide dismutase mimetics and catalase (hydrogen peroxide scavenger) inhibited brain edema formation[53,54]; antibiotic therapy prevented, in part, the oxidative stress in experimental pneumococcal meningitis[55]. Brain resident cells produce O_2^-, H_2O_2 as part of the host immune response to invasive bacterial infection, in addition, *S. pneumoniae* itself is also an important source of H_2O_2, which is not only able to cause direct cytotoxic damage but also reacts with the host's NO to form the highly reactive species ONOO[56]. Peroxynitrite can crosses membranes, activate the MMPs, leads to DNA damage, protein carbonylation and cause lipid peroxidation[52], leading to a membrane integrity loss, energy depletion, contributing to cell injury during pneumococcal meningitis[57].

NEURONAL DAMAGE AND TARGETS FOR ADJUNCTIVE THERAPY

Pneumococcal meningitis causes sequelae including sensory-motor deficits, hearing loss, deafness and neuro intellectual impairment, including deficits in learning and memory. These neurofunctional consequences occur in up to 30% of survivors patients[5]. The neuronal damage is caused by the strong inflammatory reaction and direct effects of the microorganism[32]. Significant injury during bacterial meningitis arises from mechanisms of neuronal apoptosis, particularly in the hippocampus; in autopsies cases of bacterial meningitis were found apoptosis of neurons in the dentate gyrus[58]. Apoptosis can involve both the caspase-dependent and the caspase-independent pathway[59]. The caspase-independent pathway is triggered by the pneumolysin and H_2O_2 that are produced by *S. pneumoniae*. The actions of these toxins result in an increase in ROS and calcium, resulting in mitochondrial dysfunction that leads to the release of apoptosis-inducing factor into the cytosol[56,59]. However, the caspase-dependent pathway occurs later, and pneumococcal cell-wall components trigger the required host inflammatory response from the leukocytes. p53 tumor suppressor protein and ATM protein kinase (ATM) as upstream mediators that converge on the mitochondria to initiate the release of cytochrome c, which is necessary to form the apoptosome forming apoptotic protease activating factor-1 (Apaf-1) and active caspase-9 that results in the activation of caspase-359.

In the pre-antibiotic era when acute bacterial meningitis was described the mortality rate was from 90 to 100%. Although, nowadays, with the development of highly effective antibiotics, more precocity in diagnosis with precise diagnostic methods as immunological and molecular biology methods, supportive care in intensive care units the mortality doesn't decrease. The mortality of *S. pneumoniae* is (16-37%), *N. meningitidis* (5%), *Haemophilus influenzae* (3%). This maintenance of mortality is explained by inflammation in the subarachnoid space caused by the generation of bacterial cell wall components in CSF during treatment of meningitis with antibiotics[2,60,61]. Bacterial cell wall components stimulate the release of inflammatory cytokines in the CNS such as TNF-α, IL-1β, and prostaglandins.

Possible therapeutic approaches to decrease the harmful effects of TNF and/or IL-1-β are investigated *in vitro* and animal model and might include: a) Drugs or procedures to decrease their production, block their biologic activity or enhance removal from the circulation; b) Passive immunization with antibodies against TNF and IL-1, limitation is the BBB even during inflammation; c). Drugs that interfere with IL-1 induced arachidonic acid metabolites.

Corticosteroids are largely used as adjunctive therapy for acute bacterial meningitis since the 90 decade.

In addition to corticosteroids, several other adjunctive approaches may be useful (Table)[60-67]; although there is no proved clinical evidence for use of these therapies and some are only experimental. The use of bactericidal but nonbacteriolitic antibiotics to reduce endotoxin and other injurious substance release into CSF as rifampicin and daptomycin have been investigated in animal models of pneumococcal meningitis[63]. Thalidomide acts by blocking TNF release from microglia[65]. An enhanced lifespan of activated neutrophils in the CSF contributes to massive leukocyte accumulation and host-driven cytotoxicity. Roscovitine, a purine derivative

that induces apoptosis in neutrophils, is studied in mouse models. TNF-α inhibitor decrease TNF-α levels and pleocytosis in CSF[68], blocking IL-6 intravenously in a rat model of pneumococcal meningitis reduced also pleocytosis[69] and TGF-β2 administrated intraperitoneally reduced the subarachnoid inflammation in rats with pneumococcal meningitis[54]. Intracisternal administration of the caspase-3 inhibitor reduced apoptosis in the dentate gyrus hippocampal[70] and blocking of caspase-1 demonstrated lower levels of IL-1β[71].

Corticosteroids are highly effective in reducing IL-1β production *in vitro* and *in vivo*. A meta-analysis that included all double-blinded, randomized, placebo-controlled trials since 2001 that evaluated dexamethasone in bacterial meningitis concluded that adjunctive dexamethasone in bacterial meningitis may reduce mortality and hearing loss for patients in developing countries. There may be no benefit for individuals in developing nations with a high prevalence of HIV infection[66]. The intravenous dose of dexamethasone proposed is 2 x 0.4 mg/kg/24h or 4 x 8-12mg/24h, during 48 to 96 hours. The first dose was delivered prior or concomitantly with the first dose of parenteral antibiotic[67]. In previous studies we verified dexamethasone treatment reverses cognitive impairment but increases brain oxidative stress in rats submitted to pneumococcal meningitis[72]. Adjunctive dexamethasone in bacterial meningitis reduces mortality and hearing loss for patients but still needs more evaluation.

Table. Adjunctive therapy for acute bacterial meningitis.

Corticosteroids
Nonbacteriolytic antibiotics
Nosteroidal anti-inflammatory agents
Prostaglandin inhibitors
Anti-endotoxin-biding agents
Monoclonal antibodies directed against endotoxin, cytokines or leukocyte-endothelium adhesion molecules
Pentoxifylline
Cytokine antagonists
NOS inhibitors
Thalidomide
Osmotic dehydrating agents (manitol, glycerol)
Scavengers of peroxynitrite
MMPs Inhibitors
Adenosine A_{2A} receptors agonists
Reducing lifespan of neutrophils

NOS: nitric oxid synthases; MMPs: matrix metalloproteinases.

FINAL REMARKS

Despite the significant advances in treatment, pneumococcal meningitis remains one of the most important infectious diseases of the CNS with high mortality and morbidity. Experimental animal model with its limitations continues to provide the understanding of this complex pathophysiology disease and propose new therapies adjunctive.

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