Pathophysiology of Bacterial Meningitis: Mechanism(s) of Neuronal Injury

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No bacterial disease has undergone a more dramatic change in epidemiology during the past decade than acute bacterial meningitis. This review describes the changing epidemiology and considers some important recent observations that contribute to our understanding of the pathogenesis and pathophysiology of meningitis. The major focus is on the mechanisms of neuronal injury and the pathophysiologic concepts responsible for death and neurologic sequelae. In recent years, experimental studies have amplified our understanding of the substantial body of evidence that now implicates cytokines and chemokines, proteolytic enzymes, and oxidants in the inflammatory cascade leading to tissue destruction in bacterial meningitis. The molecular mechanisms responsible for oxidant-induced neuronal injury in meningitis are explored in some depth. Genetic targeting and/or pharmacologic blockade of the implicated pathways may be a future strategy for therapeutic adjunctive measures to improve outcome and may hold substantial promise, in concert with antimicrobial agents, in humans with acute bacterial meningitis.

From the first description of meningococcemia and meningococcal meningitis, before identification of the etiologic organism (by Vieusseux in 1806) until the early twentieth century, bacterial meningitis was considered a nearly uniformly fatal disease, although some patients with meningococcal meningitis survived without antimicrobial therapy. The advent of various experimental procedures followed by antisera therapy directed against meningococci reduced mortality rates during World War I, but the introduction of sulfonamides and penicillins along with other antimicrobial agents heralded the reduction in morbidity and survival possible with modern antimicrobial therapy. Despite these achievements and the introduction of several new antimicrobial agents with in vitro activity against the major meningeal pathogens plus some limited improvement in diagnostic assays, bacterial meningitis remains associated with unacceptably high morbidity and mortality [1].

Endemic meningitis is relatively unusual in developed countries although epidemic meningitis occurs with considerable frequency in resource-limited settings. Explosive epidemics of meningococcal meningitis have continued to affect the so-called meningitis belt of sub-Saharan Africa. The most recent epidemic, which began in 1996, has resulted in over 300,000 cases and 30,000 deaths [2]. At present, exclusive of epidemics, about 1.2 million cases of bacterial meningitis are estimated to occur annually worldwide with 135,000 deaths [3]. Bacterial meningitis is now a “top 10” infectious cause of death worldwide, and about half the survivors have neurologic and other sequelae of the disease [4].

Over the past four decades, clinical and neuropathologic studies have documented the clear association between bacterial meningitis and brain edema formation, impairments of cerebrospinal fluid (CSF) hydrodynamics, increased intracranial pressure, seizure activity, arterial and venous cerebral vascular insults (that may relate to death), and other neurologic sequelae. Therefore, during the past 15–20 years, emphasis has been on a greater understanding of the pathogenesis and pathophysiology of bacterial meningitis in an attempt to improve outcome [5].

It is apparent that the host immune response is incapable of controlling infection within the central nervous system (CNS), particularly the CSF within the subarachnoid space, and that this host inflammatory response may be responsible for many adverse events during bacterial meningitis [6, 7]. 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. This has resulted in the search for adjunctive therapies for bacterial meningitis, including corticosteroids.

In this brief review, we highlight recent epidemiologic trends in bacterial meningitis worldwide and consider the current concepts of the pathogenesis and pathophysiology of this syndrome with an emphasis on the identification of promising targets for adjunctive therapy. Some of these concepts have been reviewed recently [8–10]. The pathogenesis of bacterial meningitis, including colonization of the nasopharynx by the pathogen, microbial invasion into the intravascular space and survival within the bloodstream, microbial entry mechanisms into the CNS, survival within the subarachnoid space, and host and/or en-
environmental factors contributing to susceptibility to invasive disease are reviewed more extensively elsewhere [8].

Changing Epidemiology of Acute Bacterial Meningitis

No bacterial infection worldwide has undergone a more spectacular evolution in epidemiology than acute meningitis since 1990. The introduction of *Haemophilus influenzae* conjugate vaccines (Hib) in the United States and western Europe has resulted in a decline in the incidence of invasive *H. influenzae* infections by $\geq 90\%$ [1]. As a result, *Streptococcus pneumoniae* and *Neisseria meningitidis* are the major causes of bacterial meningitis in these regions. Furthermore, a dramatic change in the epidemiology has resulted from these achievements such that the median age of onset of acute bacterial meningitis has increased from age 9 months to age 25 years in the United States [1]. Similarly, the availability of conjugate pneumococcal vaccines has led to the near total elimination of invasive pneumococcal disease, including bacteremia and meningitis, in young children in regions adopting this approach. Nevertheless, most countries in developing regions have not added the Hib vaccine to the standard vaccine repertoire offered to children, because of cost. As a result, an estimated 350,000–700,000 children worldwide still die of invasive Hib disease each year.

In 1999, the Global Alliance for Vaccines and Immunization (GAVI) was formed with the mission to ensure adequate vaccination of every child worldwide against preventable diseases. Within 18 months of its inception, GAVI committed more than $800 million to the vaccine fund in 36 developing countries over 5 years to foster deployment of vaccines such as Hib in susceptible populations (http://www.vaccinealliance.org). While the ultimate effect of pneumococcal conjugate vaccines on the epidemiology of bacterial meningitis is not known, it is hoped that uniform vaccination, such as recently introduced into the United States, will nearly totally eliminate this form of meningitis, which is associated with the highest mortality and morbidity rates. Again, cost will be an issue in resource-limited settings.

According to criteria set forth in the 1992 Institute of Medicine report on emerging infectious diseases, meningococcal meningitis and meningococcemia are emerging infectious diseases [11]. The interdependence of the worldwide community and travel have enabled the spread of meningococcal strains from the Indian subcontinent to Saudi Arabia during the Haj to the sub-Saharan African meningitis belt and the onset of explosive epidemics. Clusters of meningococcal disease have also been reported from multiple regions in recent years [12, 13]. Clusters are defined as “two or more clinical or confirmed cases of meningococcal disease arising within 12 months in the same defined setting—household, nursery/playground, primary school, secondary school, university or college” [11, 13]. This phenomenon has been reported in North America and Europe following the introduction of new serotypes of serogroup C (particularly serotype C:2a:P1.2) into a population in which serogroup B strains had been the predominant causes of endemic disease.

As documented in Scandinavia, susceptibility to invasive meningococcal disease is largely due to acquisition of a new virulent strain in a host without circulating bactericidal antibody against the causative organism [14]. The United Kingdom in November 1999 was the first country to launch vaccination against group C meningococcal disease, and a rapid decrease in the incidence of this syndrome (including clusters) is expected (see Public Health Laboratory Service [UK] annual report 1988–1999 and highlights 1999/2000). Infants and young adults aged 15–17 years are targeted, and this strategy may be applicable to other regions with clusters of meningococcal disease. Serogroup A (+/−C) vaccines are currently available. Widespread use of these vaccines in the meningitis belt is warranted to reduce morbidity and mortality while more complex polysaccharide-protein conjugate vaccines are phased into use.

Another disturbing epidemiologic trend is the emergence of antimicrobial resistance among meningococcal pathogens. At present the most serious is the emergence of penicillin resistance (along with resistance to multiple other antimicrobial agents) among *S. pneumoniae*. Antimicrobial usage, particularly β-lactams in low concentrations for prolonged periods, leads to selection for resistance among pneumococci in children and adults—both the carrier state and invasive disease; nevertheless, penicillin resistance varies widely among developed countries [15]. In addition to this selective pressure, other factors contribute to the development and spread of resistant pathogens, including an extension of their spectrum of resistance and resistance genes among diverse microorganisms and mutations in common genes [16].

Resistance to penicillin involves mutations in 1 or more penicillin-binding proteins (PBP) in *S. pneumoniae*, reducing the affinity for penicillin and related antibiotics. These mutations are usually present in the transpeptidase penicillin-binding domain. Multiple mutations are required to result in high-level resistance among PBP variants [17]. The genes that encode for the altered PBPs are called “mosaics,” since they consist of native pneumococcal DNA mixed with fragments of foreign DNA, presumably derived from streptococci normally resident in the healthy human nasopharynx. This foreign DNA has been incorporated by the pneumococci into the chromosome. The worldwide spread of penicillin resistance among *S. pneumoniae* appears to be due to dissemination of several clones carrying altered PBP genes. In addition, chloramphenicol resistance has appeared among meningococci. If this trend continues on a worldwide basis, the consequences will be devastating as chloramphenicol is widely used as the antimicrobial of choice in resource-limited settings, especially in the sub-Saharan African meningitis belt. Penicillin resistance among meningococci is also spreading (e.g., from $\sim 9\%$ of strains in 1998 to 25% of strains...
in 2001 in Ontario, Canada), but the impact on therapeutic strategies is not known.

The clinical outcome of acute bacterial meningitis depends on multiple factors including the socioeconomic status of the patient (developed vs. developing countries), age, pathogen, and the clinical characteristics and laboratory manifestations of the acute infection. For example, the case fatality rate for *H. influenzae* type B meningitis in recent reviews has been about 5% [1], a figure similar to that of meningococcal meningitis in the same report. Conversely, the mortality rate associated with pneumococcal meningitis is about 20% versus 15%–30% for *Listeria monocytogenes* in recent years [1]. About 30% of the survivors of pneumococcal meningitis develop long-term sequelae such as hearing loss, neuropsychologic impairment, and neurologic deficits [18].

The case fatality rate of *S. pneumoniae* meningitis varies dramatically by age—with a case fatality rate of <10% in children but about 40% in those ≥50 years old. These results apply only to developed countries. In developing regions, 60%–80% of children and adults with pneumococcal meningitis still succumb to the infection [18, 19]. The risk of death is greatest for those with neurologic complications during the acute illness (e.g., cerebrovascular insults, hydrocephalus, brain edema, and involvement of large intracranial blood vessels). Brain infarction with severe irreversible cerebral damage and an increase of intracranial pressure result from cerebrovascular involvement of both arteries and veins. These complications may be diagnosed by computerized tomography, magnetic resonance imaging or angiography, or transcranial Doppler sonography, but these modalities are not widely available in resource-limited settings.

**Pathogenesis of Bacterial Meningitis**

While not the focus of this review, recent studies have enhanced our understanding of the pathogenesis of bacterial meningitis among the major pathogens. To cause disease, the pathogen must, in the absence of a neurosurgical procedure or CSF leak, colonize the nasopharynx, traverse the nasopharynx into the bloodstream, survive host defense mechanisms in the intravascular space, invade the blood-brain barrier (BBB), and survive and replicate in the subarachnoid space, producing disease. Multiple interactions between the host and the organism have been described in recent years and it is of interest that the major meningeal pathogens have several similarities in common (e.g., asymptomatic carriage in the nasopharynx, the ability to cause devastating disease, phosphoryl choline moieties, polysaccharide capsules, IgA proteases, and certain physiologic features such as phase variations, DNA transformation, and autolysis) [20–22].

Our laboratories have concentrated on *S. pneumoniae*; elsewhere in this issue the elegant work of Kim [23] on the traversal of *Escherichia coli* across the BBB is reported. A few highlights of recent studies follow (for a more detailed discussion of pathogenesis, see [8]).

**Pathophysiology of Bacterial Meningitis**

Various bacteria including the major meningeal pathogens (e.g., *S. pneumoniae*) undergo autolysis under harsh conditions such as exposure to antimicrobial agents and/or growth to stationary phase. Autolysis consists of self-digestion of the cell wall by peptidoglycan hydrolyses termed autolysins. At least 3 autolysins are recognized in pneumococci, but the major autolysin is the N-acetyl-muramoyl-l-alanine amidase (LytA) [24]. Activation of LytA and autolysis result in the release in subcapsular bacterial components including peptidoglycan, lipoteichoic acid, bacterial DNA, and pneumolysin.

**Mechanisms of immune activation.** Various cell wall products of meningeal pathogens are well-known inducers of the inflammatory host response. The inflammatory response in the subarachnoid space characteristic of acute purulent meningitis can be reproduced by the intracisternal challenge with whole heat-killed unencapsulated pneumococci, their isolated cell walls, lipoteichoic acid, or peptidoglycan, but not by the injection of heat-killed encapsulated strains or isolated capsular polysaccharide [25, 26]. Exact mechanisms of immune activation by pneumococcal cell wall products remain poorly understood, but recent in vitro studies suggest that the first step in immune activation is binding of peptidoglycan and/or lipoteichoic acid to the pattern recognition receptor membrane CD14 (mCD14). mCD14 is not a transmembrane molecule and thus by itself cannot transmit the activating signal into the cell. A second step in immune activation is necessary and this potentially occurs through the toll-like receptor-2 (TLR-2).

Coexpression of CD14 and TLR-2 in Chinese hamster ovary fibroblasts confers responsiveness to pneumococcal peptidoglycan and heat-killed *S. pneumoniae* as evidenced by inducible translocation of the nuclear transcription factor NF-κB [27]. These studies also suggest that pneumococci stimulate both a TLR-2–dependent and a TLR-2–independent pathway; however, in a model of pneumococcal meningitis, TLR-2–deficient mice responded to live pathogens virtually to the same extent as wild type mice (Koedel et al., unpublished data). Leukocyte infiltration into the subarachnoid space and brain mRNA expression of proinflammatory cytokines and chemokines did not differ between TLR-2–deficient and wild type mice inoculated with live pneumococci. It appears that mechanisms other than binding of pneumococcal cell wall products to TLR-2 play a central role in the induction of the host immune response during pneumococcal meningitis. It appears that both TLR-2–dependent and –independent (pneumolysin) pathways are sufficient to cause inflammation in the absence of the other, but in vivo both are likely activated.

TLR-2–independent immune activation may be mediated at least in part by the pneumococcal toxin pneumolysin. Pneu-
molydin stimulates the production of inflammatory mediators in vitro including tumor necrosis factor (TNF-α), interleukin (IL)-1β, and IL-6 [28]. Pneumolysin is also an inducer and/or activator of enzymes such as phospholipase A2, COX-2, and inducible nitric oxide synthase (iNOS). However, in a rabbit meningitis model, a pneumolysin-deficient pneumococcal strain resulted in an inflammatory response similar to that induced by injection of the wild type strain, suggesting that pneumolysin is not essential for the induction of meningeal inflammation [29].

Another potential trigger of immune activation during acute meningitis is bacterial DNA released during bacterial autolysis. Bacterial DNA has substantial immune stimulatory effects on B, NK, and dendritic cells and on monocytes and macrophages [30, 31]. The activity of bacterial DNA is mediated by unmethylated CpG motifs, in particular base contexts. In fact, when mice or rats are injected intracerebrally with bacterial DNA or unmethylated CpG oligonucleotides, meningitis developed within 12 h. Bacterial DNA appears to initiate CNS inflammation by stimulation of macrophages and proinflammatory products such as TNF-α [32]. TLR-9 is absolutely required for cellular responses to CpG DNA, and TLR-9–deficient mice show no response to CpG DNA (inflammatory cytokine production from macrophages, maturation of dendritic cells, or proliferation of splenocytes) [33]. TLR-9–deficient mice are also completely resistant to the lethal effects of CpG DNA without production of serum proinflammatory cytokines. Thus, the role of the CpG DNA–TLR-9 pathway for immunity activation in acute bacterial meningitis deserves further evaluation. In summary, subcapsular bacterial components act as inducers of the host inflammatory response in acute bacterial meningitis, but the host receptor systems and downstream elements of signal transduction are still largely unexplored.

**Intracellular signal transduction pathways.** For meningeal pathogens, the major inflammatory stimuli are lipopolysaccharide (LPS) and peptidoglycan for gram-negative and gram-positive organisms, respectively. These inflammatory stimuli activate IκB kinase NF-κB pathways and 3 mitogen-activated protein kinase (MAPK) pathways: extracellular signal-regulated kinases (ERK) 1 and 2, c-Jun N-terminal kinase (JNK), and p38 [34, 35]. As a result of activation of these signaling pathways, a variety of transcription factors are activated: NF-κB (p50/p65) and activator protein-1 (cFos/cJun), which coordinate the induction of many genes encoding a variety of inflammatory mediators [36].

Soluble peptidoglycan strongly activates ERK-1 and -2 in the mouse macrophage cell line RAW264-7 and moderately activates JNK, while weak activation of p38 MAPK is observed. In contrast, LPS strongly activates all of these kinases, suggesting a similar but not identical activation of signal transduction pathways by these major inflammatory mediators of meningeal pathogens [37].

Pneumococcal cell wall components release inflammatory mediators by mouse microglia, which is dependent on both p38 and ERK-2/ERK-1 MAPK activities [38]. These same MAPKs are also activated when rat or human astrocytes are stimulated with various pneumococcal cell wall fragments. In addition, pneumococci activate NF-κB in undifferentiated human and mature murine monocytes [39]. Although the signaling pathways involved in immune inactivation only recently became a major focus of research activity, a rat model of pneumococcal meningitis showed a marked increase in NF-κB activity. Pharmacologic inhibition of NF-κB led to a significant reduction of host inflammatory responses as evidenced by lower CSF leukocyte and IL-6 concentrations [40]. It appears that NF-κB is a central transcriptional activator of many genes that encode proteins, host factors, or both involved in the pathophysiology of pneumococcal meningitis, including cytokines, chemokines, and adhesion molecules.

**Proinflammatory cytokines.** Multiple cytokines play an important regulatory role in the control of inflammation. TNF-α, IL-1β, and IL-6 are major early response cytokines that trigger, often in synergy, a cascade of inflammatory mediators, including other cytokines, arachidonic acid metabolites, chemokines, and reactive nitrogen and oxygen intermediates [41]. Multiple cell types within the CNS including cerebrovascular endothelial cells, astrocytes, and microglia produce all 3 cytokines. Increased concentrations of these cytokines have been detected in CSF samples from patients with acute bacterial meningitis and concentrations of IL-1β, but not IL-6 and TNF-α, are associated with significantly worse disease outcome or disease severity [42].

Brain mRNA and protein expression of IL-1β, IL-6, and TNF-α are markedly up-regulated in rodents during the acute stage of experimental pneumococcal meningitis [43, 44]. Recent studies with genetically engineered mice provide further insight into the role of acute phase cytokines in the pathophysiology of pneumococcal meningitis. For example, brain bacterial titers were not affected by either TNF-α or TNF receptor deficiency in murine models of CNS infection. Furthermore, leukocyte recruitment into the subarachnoid space was not affected by TNF deficiency. In contrast, mice with targeted disruption of TNF receptors p55 and p75 had decreased meningeal inflammation [45]. It appears that other TNF receptor ligands (e.g., lymphotoxin-α) must contribute to the induction and/or maintenance of the inflammatory response during pneumococcal meningitis. Intracisternal inoculation of pneumococci caused a three-fold increase in CSF leukocyte concentrations in IL-6–deficient mice when compared with wild type controls. Increased CSF pleocytosis is thought to result from higher brain expression of the potent neutrophil chemoattractant macrophage inflammatory protein (MIP)-2 [8]. These observations are similar to those in animal models of endotoxin-induced lung injury and endotoxemia, where IL-6 appears to control the extent of the inflammatory response by down-regulating the expression of chemokines and/or proinflammatory cytokines.

No data have been presented on IL-1β or IL-1 receptor de-
iciency and the pathophysiology of acute bacterial meningitis. The role of caspase (Casp)-1 in the pathophysiology of pneumococcal meningitis has been the subject of recent experiments. Casp-1 plays a central role in the generation of mature IL-1β and IL-18 [46]. Casp-1 mRNA and protein expression is up-regulated in the brain during experimental pneumococcal meningitis; this up-regulated activation is associated with increased levels of IL-1β [8]. Furthermore, depletion of the Casp-1 gene and/or pharmacologic blockade of Casp-1 attenuates the meningitis-induced increase in IL-1β. A significant reduction in NF-κB activity, brain TNF-α, MIP-1α, and MIP-2 expression, and CSF pleocytosis was also observed [8]. The Casp-1–IL-1β signaling pathways play a crucial role in the induction and amplification of the host inflammatory response during pneumococcal meningitis. TNF-α and IL-1β also stimulate the expression of chemokines and adhesion molecules, which further facilitate the passage of leukocytes from the circulation into the subarachnoid space.

Chemokines. Based on the number and spacing of the conserved N-terminal cysteines, chemokines are currently subdivided into 4 distinct subfamilies: the CXC or α, CC or β, CX3C or γ, and C or δ subfamilies [47]. The CXC chemokines are further divided into 2 groups depending on the presence of the ELR motif preceding the first cysteine. Despite this classification, within each chemokine subfamily the individual members often display overlapping chemoattractant activity. ELR-CXC chemokines, such as IL-8 and growth related protein (Gro)-α, are effective chemoattractants for neutrophils, but not for monocytes. In contrast, non–ELR-CXC chemokines, such as IP-10 and, CC chemokines, such as monocyte chemotactic protein (MCP)-1 and MIP-1α, are poor chemoattractants for neutrophils but potent attractors of monocytes and lymphocytes.

Elevated levels of the chemokines IL-8, Gro-α, MCP-1, MIP-1α, and MIP-1β are found in human CSF during bacterial meningitis [48]. Furthermore, CSF from patients with bacterial meningitis is chemotactic for neutrophils and mononuclear leukocytes in an in vitro chemotaxis assay. Neutrophil chemotaxis is reduced by anti–IL-8 and anti–Gro-α antibodies, and mononuclear cell migration is also reduced by a combination of anti–MCP-1, anti–MIP-1α, and anti–MIP-1β antibodies [48]. Brain mRNA and protein expression of MIP-2 and KC, the two functional homologs of human IL-8, are markedly increased during the acute phase of pneumococcal meningitis in a murine model [43, 44]. Neutralization of MIP-2 bioactivity reduced neutrophil influx into the CSF in an infant rat model of Hib meningitis [8]. Thus, the available evidence suggests that ELR-CXC chemokines play a crucial role in leukocyte traversal into the CSF during acute bacterial meningitis.

Leukocyte migration into the subarachnoid space. Leukocyte migration from the circulation into the extracellular space occurs through a multistep process governed by the sequential activation of adhesion receptors and their ligands on both leukocytes and the endothelium [49]. Four sequential steps (tethering, triggering, firm adhesion, and emigration) are involved in this adhesion cascade. The selectin family of adhesion molecules (P-, E-, L-selectin) mediates tethering and promotes leukocyte rolling under flow conditions.

Firm adhesion of leukocytes to the endothelium is mediated by a family of integrins. Integrin activation requires a triggering step mediated by a proinflammatory cytokine (e.g., IL-1β) and chemokines (e.g., IL-8), complement products, and bacterial cell wall components. Leukocyte integrins, once activated, bind to endothelial cell count receptors. Macrophage antigen 1 (MAC-1; CD11b/CD18) is the predominant integrin involved in neutrophil binding to the activated endothelial cell. Intercellular adhesion molecule (ICAM)-1, an immunoglobulin-like molecule, exhibits low constitutive levels on the cell surface of the resting endothelium but is markedly induced by exposure to inflammatory stimuli and is the most important endothelial ligand for MAC-1. After firm adhesion to endothelial cells, leukocytes migrate along a chemotactic gradient, mediated by chemokines into the subarachnoid space.

Partial inhibition of CSF leukocyte accumulation in the subarachnoid space was noted in P-selectin–deficient mice during experimental meningitis, whereas mice doubly deficient in P- and E-selectins showed dramatic and nearly complete inhibition of leukocyte accumulation into the subarachnoid space [50]. Antibodies directed against the adhesion molecules MAC-1 or ICAM-1 also reduced influx of neutrophils during experimental meningitis and led to significant reductions in intracranial complications such as brain edema formation [51].

Mediators of brain damage. As shown in figure 1, by following the steps enumerated above, activated leukocytes can release potentially tissue destructive agents including reactive oxidants and proteolytic enzymes. Matrix metalloproteinases (MMPs) have been the focus of recent investigations, both in patients with bacterial meningitis and in experimental models of the disease.

MMPs. MMPs are a family of zinc-dependent endopeptidases responsible for tissue remodeling via degradation of extracellular matrix components [52, 53]. Collagen IV and fibronectin are crucial components of the subendothelial basal lamina of cerebral microvessels and contribute to the integrity of the BBB. BBB disruption follows the intracerebral injection of MMPs in experimental models [54]. Although it is possible that migrating neutrophils release MMPs to facilitate traversal from the circulation into the subarachnoid space, evidence for this process is not available. The broad-spectrum MMP inhibitor BB-94 markedly attenuated BBB disruption but did not significantly reduce CSF pleocytosis in a rat model of meningococcal meningitis [55]. Similarly, treatment with MMP inhibitors led to a significant reduction of CSF TNF-α concentrations, BBB permeability, and the extent of neuronal injury in an infant rat model of pneumococcal meningitis [56, 57]. MMP-9 and -8 are up-regulated in the CSF during bacterial meningitis in humans, and higher concentrations of MMP-9
Figure 1. Mechanisms of brain damage in experimental pneumococcal meningitis. NF-κB, a transcriptional activator of many genes involved in the pathogenesis of bacterial meningitis, encodes host factors including proinflammatory cytokines, chemokines (e.g., interleukin [IL]-1β), and adhesion molecules. The proinflammatory cytokines IL-1β and tumor necrosis factor (TNF)-α are synthesized as inactive precursors that are processed to mature active forms by proteases (Caspa1 [Casp1], also known as IL-1β-converting enzyme, and TNF-α-converting enzyme [TACE]). IL-1β and TNF-α are potent activators of NF-κB. This process may lead to the uncontrolled expression of proinflammatory mediators and the increased expression of adhesion molecules both on the endothelium (e.g., intercellular adhesion molecule [ICAM]-1) and on neutrophils, leading to subsequent massive influx of leukocytes into the subarachnoid space. Once present, activated leukocytes release a complex variety of potentially cytotoxic agents including oxidants and proteolytic enzymes (e.g., matrix metalloproteinases [MMP]), which may contribute to tissue destruction. Also, peroxynitrite may cause brain damage via a variety of independent mechanisms. The best studied are attack of polyunsaturated fatty acids, leading to lipid peroxidation, and an alternative pathway that involves oxidant-induced DNA strand breakage and subsequent poly (ADP ribose) polymerase (PARP) activation, which initiates an energy-consuming intracellular cycle that ultimately results in cellular energy depletion and cell death. Both mechanisms likely contribute to cell injury during pneumococcal meningitis. ECM, extracellular matrix; MIP, macrophage inflammatory protein.

are associated with neurologic deficits in patients with disease compared with persons who had recovered fully, suggesting high CSF concentrations of this MMP may significantly increase the risk for adverse outcome of bacterial meningitis in humans [58].

Oxidants. In addition to proteolytic enzymes, several other major effector molecules are available to the phagocyte that may influence pathophysiology of meningitis including reactive oxygen species (ROS; e.g., superoxide) and reactive nitrogen intermediates (RNI; e.g., NO). ROS clearly play an important role in the pathophysiology of acute bacterial meningitis. For example, ROS generation has been detected in brain sections of infant rats with group B streptococcal meningitis by use of the manganese/diamino benzidine method and in vivo in a rat model of pneumococcal meningitis by use of the lucigenin-enhanced chemiluminescence technique [59, 60].

Meningitis-associated intracranial complications (including cerebral hypoperfusion) increase intracranial pressure, brain edema formation, and neuronal injury in experimental models of bacterial meningitis and are attenuated or even prevented by the use of antioxidants [61, 62]. NO/nitrite levels in the CSF during meningitis are elevated in humans with bacterial meningitis and in experimental animal models. NO production has been observed uniformly in animal models of bacterial meningitis, although administration of NOS inhibitors in experimental models has yielded contradictory results ranging from amelioration to deterioration of CNS complications and brain damage [63, 64]. Unfortunately, NOS inhibitors are not selective for a single isoform of NOS and may have additional pharmacologic effects unrelated to the NO pathway, thus contributing to these contradictory results.

Knockout mice with a targeted disruption of single NOS isoforms have been useful in defining the role of NO from various enzymatic sources in the CNS [65, 66]. Mice deficient in the inducible NOS isoform exhibit significantly lower levels of inflammatory mediators such as IL-1β, TNF-α, and MIP-2 than wild type mice after challenge with pneumococci. Meningitis-induced BBB disruption is significantly reduced in parallel [43]. When mice with the endothelial NOS knockout were used in similar experiments, aggravation of meningitis-induced BBB permeability was observed. It appears that brain expression of proinflammatory host factors such as IL-1β, KC, MIP, and P-selectin may be responsible for these effects [8, 44]. It is highly likely that NO generated by separate isoforms may have different roles and potentially opposing effects during pneumococcal meningitis. Inducible NOS seems to contribute to some of the harmful pathophysiologic changes of the disease while endothelial NOS-derived NO may play a protective role, potentially through inhibition of leukocyte endothelial interactions and the production of cytokines and chemokines.

Peroxynitrite is induced by various forms of inflammation and ischemia/reperfusion injury and may play a central role in bacterial meningitis from generation of oxygen and nitrogen-
centered free radicals. Nitrotyrosine concentrations, a useful marker for the formation of peroxynitrite, which lasts only seconds, were elevated in the CSF of patients with acute bacterial meningitis when compared with noninfected controls [67]. In addition, elevated concentrations of CSF nitrotyrosine are associated with an unfavorable clinical outcome. Nitrotyrosine residues on proteins have been detected immunohistochemically in leukocytes in the subarachnoid space and in other areas such as the leptomeninges penetrating cortical and occasionally parenchymal blood vessels in both adult rats with pneumococcal meningitis and in humans [67]. Treatment with a peroxynitrite scavenger, uric acid, significantly attenuates meningeal inflammation and meningitis-associated complications. The strong oxidant peroxynitrite is definitely a central mediator of the pathophysiologic alterations in bacterial meningitis [8].

Molecular mechanisms of oxidant-induced brain damage. Peroxynitrite and other oxidants may contribute to the development of brain damage and neuronal injury during bacterial meningitis by a variety of mechanisms as shown in figure 1. Lipid peroxidation through peroxynitrite influence on polyunsaturated fatty acids can lead to the loss of cellular membrane function and integrity. Malondialdehyde and 4-hydrox-2 (E-Nonenal 4-HNE), markers of lipid peroxidation, are found in elevated concentrations in both patients with bacterial meningitis and in brain homogenates of infant rats with group B streptococcal or pneumococcal meningitis [68, 69]. 4-HNE is found in inflammatory cells and in pial and penetrating cortical blood vessels by immunohistochemistry techniques. Aminooxidases, inhibitors of lipid peroxidation, attenuate the pathophysiologic alterations and neuronal injury in rat models of pneumococcal meningitis [70]. Clearly, lipid peroxidation in meningitis is associated with brain injury. A similar staining pattern for 4-HNE and nitrotyrosine suggests that RNI act as initiators of lipid peroxidation during the disease.

Results of experiments by a group in Munich suggest that another alternative pathway may explain oxidant-induced damage during meningitis via DNA strand breakage in subsequent poly (ADP ribose) polymerase (PARP) activation that initiates an energy-consuming intracellular cycle that ultimately results in cellular energy depletion and cell death [71, 72]. ADP ribose polymer formation is a well-accepted marker for PARP catalytic activity. Poly ADP-ribosylated proteins are higher in mouse and rat brains 24 h after pneumococcal inoculation than in uninfected control animals [73]. Knockout mice lacking the PARP-1 gene are protected against the development of meningitis-associated complications as outlined by the Munich group. Inflammatory host response is reduced in parallel. Pharmacologic inhibition of PARP improves the clinical course of pneumococcal meningitis. On balance, PARP activation in the brain appears to play a crucial role in the pathophysiology of bacterial meningitis [8, 73].

The vicious cycle of pathophysiologic alterations. Figure 2 shows that the combination of lipid peroxidation and PARP activation may contribute substantially to endothelial cell injury during bacterial meningitis. Once endothelial dysfunction occurs, the consequences include cerebrovascular autoregulation loss, loss of CO₂ reactivity of cerebral vessels, and increased permeability of the BBB. BBB permeability disruption allows plasma constituents to enter the brain, resulting in vasogenic cerebral edema and an increase in intracranial pressure.

Intracranial pressure is a multifactorial process in meningitis and is related not only to vasogenic edema but also cytotoxic edema resulting from leukocyte infiltration, interstitial edema resulting from blockade of normal CSF pathways, and increased blood volume in the brain [74, 75]. Marked increases in intracranial pressure can be deleterious in patients with bacterial meningitis by causing cerebral herniation or decreasing cerebral perfusion (a reduction in cerebral perfusion pressure and/or loss of cerebrovascular autoregulation) and can ultimately lead to irreversible brain injury or death.

Conclusions

Meningitis-associated brain injury and neuronal death is not mediated simply by the presence of viable bacteria but occurs as a consequence of the host reaction to bacterial components. A wide variety of inflammatory host factors involved in the complex pathophysiologic cascade of bacterial meningitis have been identified during the past decade, largely with experimental studies, but have also been confirmed in limited studies of human material. The crucial role of the caspase 1–IL-1β pathway in the induction and amplification of the host inflammatory response during pneumococcal meningitis may lead to the identification of new potential therapeutic adjunctive agents. Similarly, peroxynitrite and other important effector molecules contributing to BBB disruption and neuronal injury may be susceptible to adjunctive treatments.

Lipid peroxidation and the activation of PARP-1 are also
central mechanisms of oxidant-induced brain damage, and pharmacologic interference with these biochemical pathways may be the most promising adjunctive therapeutic strategy (reviewed in [8]). We believe that further exploration of these pathways will not only be relevant to the development of therapeutic adjunctive strategies in bacterial meningitis but may also improve outcomes in other inflammatory conditions of the CNS such as meningoencephalitis, subarachnoid hemorrhage-induced vasospasm, and ischemia reperfusion following stroke. Antimicrobial agents that modulate the release of proinflammatory bacterial compounds may also have a potential effect on bacterial meningitis as reviewed recently [76], but systematic study of this approach in humans has not yet been validated.

References

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