# Brains, bacteria and behaviors: the role of interferon-gamma in the pathogenesis of pneumococcal meningitis

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Pneumococcal meningitis is a highly lethal form of bacterial meningitis that occurs following brain infection by the Gram-positive cocci *Streptococcus pneumoniae*. Not only does it cause acute mortality, but pneumococcal meningitis also accounts for the highest proportion of survivors living with neurological sequelae, including behavioral disorders, cognitive deficits, hearing loss, motor impairment and epilepsy. More than 90 distinct pneumococcal serotypes have been identified worldwide based on their capsular compositions and serological responses. Serotype replacement continually poses great challenge to costly vaccination programs in developed countries (Koelman et al., 2020), this has therefore emphasized the need to develop new treatment strategies in addition to improving vaccine coverage. Various immunomodulatory agents, such as complement system inhibitors and matrix metalloproteinase inhibitors, have been shown to improve disease severity and mortality when tested in animal models (Bewersdorf et al., 2018). Nevertheless, given the evidence of long-term cognitive deficits and behavioral problems in patients who were clinically well recovered from pneumococcal meningitis, it remains unclear how the protection at the early inflammatory stage may translate into long-term functional recovery. With this in mind, we have established an integrated approach to investigate the interplay between acute host inflammatory response and ensuing neurological deficits in a mouse model of pneumococcal meningitis in animals that survive the lethal disease due to antibiotic ceftriaxone treatment. This has enabled the identification of a nexus between the toll-like receptors (TLRs) 2 and 4, interferon-gamma (IFN-γ) and the enzyme indoleamine 2,3-dioxygenase-1 (IDO-1) that contributes to enduring neurological impairments. Here, we will highlight the findings of our systematic studies in the hope of opening avenues for future research relevant to both meningitis as well as other neurological diseases.

Preclinical research in the past decades has demonstrated that out-of-control host inflammatory responses to invading pneumococci are central to causing cerebrovascular complications that link mortality and/or ensuing neurological disorders. Hence, current concepts of the disease pathology suggest adjunctive therapeutic modulation of the host immune response in conjunction with the administration of existing antibiotic treatments may relieve the disease burden (Bewersdorf et al., 2018). Many studies have shown that a series of inflammatory cascades elicited by the invading pathogens to the central nervous system drive the deleterious consequences of pneumococcal meningitis. TLRs, such as TLRs 2 and 4, are important pattern recognition receptors (PRRs) that sense incoming bacterial components. The activation of TLR signaling pathways then triggers a series of molecular events leading to the production of a multitude of pro- and anti-inflammatory cytokines in the central nervous system to coordinate a pathogen-eradicating mechanisms. IFN-γ, interleukin (IL)-6, IL-1β, IL-8, and tumor necrosis factor are some of the pro-inflammatory cytokines, while IL-10 and IL-1 receptor antagonist the anti-inflammatory cytokines, reported in cases of pneumococcal meningitis. A well-orchestrated pro and anti-inflammatory reaction is essential for complete recovery from infectious diseases. Conversely, intense propagation of the pro-inflammatory cytokines may disrupt the homeostatic balance, which in turn leads to adverse disease outcomes.

Notwithstanding the research progress, most research thus far has largely focused on investigating acute inflammatory responses of pneumococcal meningitis to achieve remedial outcomes. Despite numerous systematic reports confirming the presence of long-term neurological sequelae among human survivors, very little effort has been put towards studying the long-term effect of the disease on preclinical models. This is understandable due to the complexity and laborious nature of conducting long-term behavioral studies and the scarcity of funding to support such research. To overcome this technical challenge, we established a murine model of neurobehavioral deficits to facilitate investigation of long-term neurological outcomes in animals that survived pneumococcal meningitis due to antibiotic treatment. This has involved the use of an automated home cage test system, the IntelliCage™, that is capable of measuring multiple behavioral and cognitive elements simultaneously and sequentially.

By subjecting mice to a 16-day test battery twice (with a 4–6 weeks interval in between), we consistently found enduring behavioral deficits and cognitive impairments in the C57BL/6J mice that survived pneumococcal meningitis as a result of treatment with antibiotic ceftriaxone. Using this system, we identified two aspects of behavioral deficits: diurnal hypoactivity and nocturnal hyperactivity. The mechanistic causes of diurnal hypoactivity are not fully understood at present. A large number of studies on bidirectional brain-immune crosstalk have identified the pro-inflammatory cytokines IL-6, tumor necrosis factor and IL-1β as pivotal mediators of sickness behaviors that include fever and fatigue as well as reduced motivation to interact with social and physical entities (Dantzer, 2009). Besides causing sickness symptoms during the acute stage of disease, an intriguing action of these cytokines is their long-lasting influence on behaviors, presumably through sensitization of depressive-like behaviors and their modulatory roles in neural plasticity and neurogenesis. A perturbed balance of these cytokines, as seen in several neurological diseases such as Alzheimer's disease and autoimmune disorders, has supported the concept that they can impart long-lasting functional and anatomical changes in the brain, leading to lifelong behavioral and cognitive alterations (Zheng et al., 2016). By contrast, we also found nocturnal hyperactivity on exploratory activities during the active dark cycle of adaptation phases. While this behavior seems to contradict that observed during the light cycle, it might result from a hippocampus-dependent behavioral disorder, as evidenced by an early study that demonstrates similar hyperactive phenotype in response to novelty or challenge situations in hippocampus-lesioned mice (Voikar et al., 2010).

Besides behavioral disorders, we also found long-term impairment in cognitive domains of test animals. Cortical necrosis and hippocampal apoptosis represent the two major contributors to neurological sequelae of bacterial meningitis. In congruence with this, we demonstrate in our experimental model long-term impaired working memory and cognitive flexibility that are presumed to be attributable to hippocampal and cortical brain injury, respectively. It is however noteworthy that given the growing knowledge about the complexity of neuronal and synaptic networks within the brain, the observed long-term behavioral or cognitive disorders will not only be caused by physical damage within a specific brain region. The dynamics of central disruption deserve scientists' attention. As with behavioral disorders, the molecular events leading to impaired neurological functions in pneumococcal meningitis remain undetermined. There is mounting evidence that the pro-inflammatory cytokines, through mechanisms such as glial activation or oxidative stress generation (Sheng et al., 2011), may induce long-term neuronal damage or change of hippocampal plasticity.

During pneumococcal meningitis, the PRRs are important ligands for the transmission of danger messages to initiate the innate host response for the elimination of pathogens. TLRs and Nod-like receptors are the two main types of PRRs activated during acute pneumococcal infection. Several studies collectively have demonstrated the involvement of TLR2 and 4 in immune defence mechanisms during acute pneumococcal meningitis. It has been shown that by ablating the TLR2/4 pathway, the central and peripheral bacterial load becomes heightened, which accompanies increased disease severity (Klein et al., 2008). Further to this, we found that the TLR2/4 axis may participate in modulating the acute host inflammatory response to pneumococcal brain infection that, in turn, partially prevents mortality and long-term neurological problems (Too et al., 2019).

Intriguingly, IFN-γ, a pleiotropic pro-inflammatory cytokine that acts as a downstream mediator of multiple immune pathways along the TLR2/4 axis, was shown to play an important role in mediating acute mortality and long-term neurological sequelae due to brain infection by pneumococci. Our initial observations showed that genetic disruption of IFN-γ improved survival during experimental pneumococcal meningitis in the absence of antibiotic treatment (Mitchell et al., 2012). Furthermore, following antibiotic treatment, pneumococci-infected IFN-γ-deficient mice were found to exhibit diminished levels of inflammatory response, bacterial load, BBB disruption and intracranial haemorrhage 2 days post-infection, as well as lessened neuronal injury 10 days post-infection compared their wild-type equivalents (Too et al., 2014a). In the long-term, ameliorated behavioral disorders and cognitive flexibility were seen in the IFN-γ-deficient mice compared to wild-

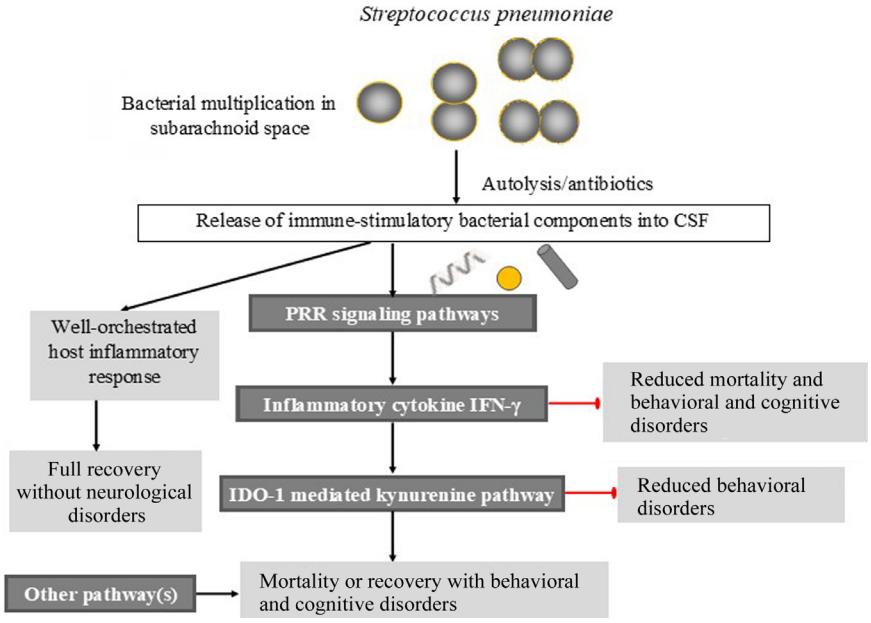


**Figure 1** | **Potential molecular mechanism that underlies post-recovery neurological sequalae due to acute pneumococcal infection.**
While the absence (red arrow with flat end) of IFN-γ alleviates mortality and neurological sequelae, inhibition of IFN-γ-induced-IDO-1 effects confers much lower degree of protection against pneumococcal brain infection, suggesting synergistic actions with other unknown pathways. A well-balanced host inflammatory response remains crucial for full functional recovery from meningitis disease. CSF: Cerebrospinal fluid; IDO-1: indoleamine 2,3-dioxygenase-1; IFN-γ: interferon-gamma; TLR: Toll-like receptor.

type equivalents. Perhaps surprisingly, genetic deletion of TLR2/4 led to elevated IFN-γ in the central nervous system, and this coincided with worsened survival and cognitive flexibility in our mouse model of pneumococcal meningitis that lacks TLR2/4 signaling pathways (Too et al., 2019). In other words, we propose that inhibition of the TLR2/4 axis leads to modulated IFN-γ production and unknown compensatory responses, and in turn this plays an immunopathological role. A decrease in IFN-γ during pneumococcal meningitis tips the balance within the host towards an attenuated inflammatory response, which protects mice against developing long-term behavioral deficits and, to a lesser extent, cognitive impairment.

IFN-γ has been implicated as contributor to neurological disorders due to inflammation-induced dysregulation of the kynurenine pathway. Therefore, to further elucidate IFN-γ-driven pathological mechanisms during pneumococcal meningitis, we examined two key enzymes of kynurenine pathways, IDO-1 and tryptophan dioxygenase-2 (TDO-2) (Too et al., 2014b). In these studies, we found only protection against behavioral disorders in IDO-1-deficient mice that survived pneumococcal meningitis following antibiotic treatment. Genetic deletion of either IDO-1 or TDO-2 does not protect mice from mortality and long-term cognitive deficits. These findings suggest that the downstream effectors of IFN-γ are multiple. Not only does the pathogenesis of mortality and morbidity only partially overlap with genetic modulation of each inflammatory mediator (TLR2/4, IFN-γ, IDO-1 and TDO-2), but also the immunopathological pathways leading to behavioral and cognitive impairments and to hearing deficits are also independent. This therefore indicates that further investigations may be needed to identify targets for individual subsets of functional impairments caused by pneumococcal meningitis. Such investigations might also reveal mediators that are central to multiple pathways, which may be targeted to alleviate the majority of impairments.

In summary, we have developed an automated behavioral testing approach in mice recovered from pneumococcal meningitis and have used this to identify specific inflammatory pathways that lead to behavioral and cognitive impairments. While not without its limitations, such as the requirement for a costly automated behavioral monitoring system, the use of single sex animals and that our studies have focused primarily on pneumococcal meningitis caused by serotype 3 pneumococci, nevertheless it is a feasible approach that allows high throughput analysis of behavioral and cognitive anomalies. Using this methodology, we have identified a series of processes that includes TLR-mediated modulation of IFN-γ production, as well as the IFN-γ dependent enzyme IDO-1, in mediating neurological deficits (**Figure 1**). In the future, advanced medical imaging technology such as animal magnetic resonance imaging may help assessing long-term neurological sequelae in preclinical non-human models. Finally, in agreement with others (Bewersdorf et al., 2018), the major implication of this work is that the use of immuno-modulatory drugs represents an important avenue for the acute treatment of pneumococcal meningitis. It is hoped that this will not only reduce fatality rate, but also long-term neurological problems.

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