Review Article

Immunopathogenesis of central nervous system fungal infections

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Fungal infections of the central nervous system (CNS) evoke humoral and cellular immune responses with the scope to enable the host to eliminate the pathogen. Immunopathogenesis of CNS fungal infections remains incompletely understood, with most of our understanding coming from studies on experimentally infected animals. However, activation of brain resident cells combined with relative expression of immunoenhancing and immunosuppressing cytokines and chemokines may play a determinant role and partially explain immunopathogenesis of CNS fungal infections.

Key words: Central nervous system, fungal infections, immunopathogenesis

CNS Fungal Infections and Pathogenesis

The incidence of central nervous system (CNS) fungal infections has greatly increased in the last few decades. However, most reports describe the immune interactions that take place in the lungs following fungal infections; in only a few reports the host pathogen interplay in the brain, the organ most frequently involved in extrapulmonary infection, has been studied. Notable is the fact that the brain is remarkably resistant to fungal infections due to the abundant blood supply and also due to the relatively impermeable blood-brain barrier. Despite the fact that the brain and subarachnoid space are protected by anatomic and functional barriers, under special conditions and immune system abnormalities fungal pathogens breach these barriers.[1] Nevertheless, the immunopathogenesis of CNS fungal infections remains incompletely studied, with most of our understanding coming from studies on experimentally infected animals.

Host defence mechanisms are known to influence the manifestation and severity of fungal infections, such that the clinical forms of the disease depend on a patient's immune response. In a host incapable of effectively clearing the primary pulmonary infection, widespread hematogenous dissemination may happen and fungal invasion of the CNS can occur. Probably, phagocytic effector cells in the brain, such as microglia and astrocytes, as well as cell-mediated immunity and cytokine release, may all play important roles in brain-specific immune response. In addition, fungal virulence factors may also modulate early signaling molecules of the host response, providing mechanisms by which these factors may up- or down-regulate cell-mediated immunity in the lung and brain.

In general, fungal invasion of the CNS may produce one or more of the following clinical syndromes: subacute or chronic meningitis, encephalitis, parenchymal brain abscesses or granulomas, stroke or myelopathy.[2] The most common pattern of the disease is basal meningitis or intraparenchymal abscesses due to fungal pathogens. Fungal diseases in the brain are usually secondary to infections elsewhere in the body, usually the lungs, less often in other extracranial sites and in the vast majority of the cases spread through the bloodstream. Intracranial seeding occurs during dissemination of the organism or only occasionally by direct extension from an area anatomically adjacent to the brain.[3]

All the major fungal pathogens can produce meningitis. On a fungus-specific basis, meningitis ranges from the relatively common cryptococcal meningitis to the rare meningitis due to dimorphic or filamentous fungi. *Cryptococcus*, *Candida*, *Aspergillus* and a series of molds can produce life-threatening CNS infections.[4,5] These infections require immediate and precise diagnosis, as well as carefully selected management approaches to optimize outcomes.

Many of the etiologic agents of fungal meningitis may also cause brain abscesses. *Candida* spp. have emerged as the most prevalent etiologic agents followed by *Aspergillus* spp., but also *Cryptococcus neoformans* and other fungi can be causative agents. In addition, many other fungi have also been reported

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to cause brain abscesses less frequently; these include *Scedosporium apiospermum*, *Paracoccidioides brasiliensis*, *Cladophialophora bantiana*, *Bipolaris hawaiiensis*, *Bipolaris spicifera*, *Exophiala dermatitidis*, *Ochroconis gallopava*, *Ramichloridium mackenzei* and *Curvularia pallescens*. Unfortunately, the diagnosis of fungal brain abscesses is often unexpected and many cases are not discovered until autopsy.[6]

In this review, cellular and molecular pathways of the immunopathogenesis of CNS fungal infections are discussed by using mostly animal data since investigations in humans are very limited.

Immunopathogenesis of CNS Fungal Infections

The role of major histocompatibility complex (MHC) and human leukocyte antigen (HLA) in CNS fungal infections

Although CNS has traditionally been regarded as an immunologically privileged site, activated T-cells can traffic across the blood-brain barrier into the CNS for immune surveillance.[7] In this way, when subjected to an injury or infection, CNS can mobilize and develop an immune response involving infiltrating $CD4^+$ and $CD8^+$ T-cells, B-cells, macrophages, neutrophils and activated resident cells such as microglia, astrocytes and endothelial cells.[8]

When a fungus activates resident cells, endogenous CNS cells express major histocompatibility complex (MHC) Class I and Class II molecules and may therefore act as antigen-presenting cells. In addition, they express complement receptors, produce cytokines, chemokines and molecules with antifungal activity, such as nitric oxide (NO) and are capable of phagocytosis.[9] Microglia, acting as antigen-presenting cells, stimulate T-cell proliferation and cytokine secretion, which in turn stimulate these semiprofessional phagocytes to ingest and more effectively kill invading fungi.

The precise mechanisms that explain the association of CNS fungal infection with the particular MHC molecules are unknown. However, several models have been proposed, including the direct involvement of human leukocyte antigen (HLA) molecules and the involvement of closely linked genes. In an immunocompetent host, during the initial immune response to a fungal pathogen, HLA molecules must bind to peptides derived from fungal proteins and the T-cell repertoire must include clones that can be activated by such HLA-bound peptides.[10] Nevertheless, non-fulfilment of either of these requirements may render a host carrying a particular combination of HLA alleles more susceptible to certain infections than another who has a different combination of alleles. Especially in CNS involvement of paracoccidioidomycosis, the MHC molecules are not expressed in a constitutional way in the CNS, at least at the level found in the majority of other tissues. In addition, in situations with an immunological stimulation there is an increase of expression of these molecules.[11]

The Role of Cytokines and Chemokines in CNS Fungal Infections

When an immune response within the CNS is initiated, amplification or suppression depends on a number of factors, including the activation state of microglia, the levels of cytokine and cytokine receptor in glial and immune cells, relative expression of immunoenhancing and immunosuppressing cytokines, the locations of these cytokines within the CNS and the temporal sequence in which a particular cell is exposed to various cytokines.[12] The actions of cytokines on the vasculature in the brain also may be of pathophysiological relevance. There is increasing evidence that a variety of cytokines such as interleukin (IL)-1α, IL-1β, IL-4, IL-6, IL-10, IL-12, IL-18, transforming growth factor-β1 (TGF-β1) and tumor necrosis factor-α (TNF-α) are constitutively expressed in the brain of animal models with CNS fungal infections. In addition, a variety of chemokines such as IL-8, macrophage inflammatory protein-1α (MIP-1α) and -1β (MIP-1β) and monocyte chemoattractant protein-1 (MCP-1) are also involved in the immunopathogenesis of CNS fungal infections. However, this cytokine/chemokine profile does not suggest a polarized Th1 or Th2 response and may illustrate that CNS fungal infection is the result of an ineffective immune response; possibly due to an insufficient antifungal effector function of endogenous glial cells resulting from competing pro- and anti-inflammatory cytokines. Important is the fact that the precise cellular origins of each cytokine remain to be determined.[13]

In an intracerebral model of local infection, expression of, as well as local exogenous administration of IL-6 or IL-1β were associated with enhanced survival.[14] A CNS-specific and TNF-α-dependent role for IL-6 and IL-1β in protection against cryptococcosis was suggested by findings with TNF/lymphotoxin-α-deficient mice. The markedly reduced survival of TNF/lymphotoxin-α-deficient mice was associated with a marked reduction in brain levels of IL-6 and IL-1β, while levels of these cytokines in plasma and other tissues were similar in knockout and parental strain mice, while levels of interferon-γ (IFN-γ) and IL-12 were higher in knockout than in parental strain mice.[15]

In patients with AIDS and meningeal cryptococcosis as well as in experimental murine cryptococcal meningoencephalitis, cytokines IL-1α, IL-1β, IL-6, TNF-α, IFN-γ, IL-4 and IL-10 and molecules with bactericidal activity, such as NO and inducible nitric oxide synthase (iNOS) were induced above baseline levels, in the brain during the course of cryptococcal infection.[7,9]

Additionally, the expression of IL-1α, TNF-α and iNOS mRNAs showed similar kinetics, with early detection on first days of infection and augmented levels at later time points. In contrast, the expression of IL-4, IL-6 and IFN-γ mRNAs was detected on later days, followed by the expression of IL-1β and IL-10 even more lately. The expression of mRNA for IL-4, IL-10 and IFN-γ remained stable in later stages of infection.

Among cytokines there are differences on the time when initial expression begins. Specifically, in an animal model, initial expression of IL-12, IL-18, TGF-β1, IL-1α, TNF-α and iNOS transcripts was detected prior to the appearance of observable infiltrating inflammatory cells suggesting that these mRNAs were produced by resident cells in CNS. By contrast, IL-1β, IL-4, IL-6, IL-10 and IFN-γ transcripts were detected in later stages postinfection.[7]

Noticeable is the fact that the concomitant expression of TGF-β1, IL-4 and IL-10 might be able to act as immunosuppresant, allowing the continuation of the infectious process. Additionally, although in the early stages of infection NO contributes to the killing of yeasts, the expression of iNOS by endogenous cells may have been modulated by the immunosuppressive cytokines or NO may cause immunosuppression itself, thereby permitting progression of the infection. The paradoxical depression of iNOS may happen in the brain as result of the neuroprotective action of microglia, expressing suppressive cytokines, such as TGF-β1, to a greater degree than proinflammatory cytokines IL-1β, IL-6, IL-12, IFN-γ and TNF-α under natural conditions.[16,17]

IL-12 is produced by monocytes, B-cells and activated microglial cells in the CNS. The role of IL-12 is to induce the production of IFN-γ by T-cells and natural killer (NK) cells and the development of a Th1 cellular immune response against infection, resulting in a positive effect on host immune response.[18] The results on the detection of IL-12 in the brain during a CNS fungal infection are contradictory. However, constitutive expression of mRNA for the IL-12p40 subunit in the brain has been found in infected mice.[7]

In a murine model of cryptococcal meningoencephalitis, mRNA levels of TGF-β1, IL-12 and IL-18, were expressed constitutively and stayed at stable levels in both infected and uninfected animals. This finding is consistent with the fact that TGF-β1, produced by endogenous glial cells, acts as an anti-inflammatory agent and contributes to normal function of CNS. In addition, the expression of IL-1α was induced during progressive *C. neoformans* infection.[17]

TNF-α, like IL-1 is a major immune response-modifying cytokine produced primarily by activated macrophages but also by macrophages, monocytes, neutrophils, T-cells and NK-cells. Cells expressing CD4 secrete TNF-α while $CD8^+$ cells secrete little or no TNF-α. Early production of TNF-α is required to prevent the establishment of cryptococcal foci in the CNS. In an animal model, TNF-α and iNOS are produced in direct response and in proportion, to the magnitude of the *C. neoformans* infectious burden seen in the brain.[7]

The role of IFN-γ has been shown to be important against *C. neoformans* CNS infections. In mice, IFN-γ was found to be essential for optimal growth inhibition when *C. neoformans* was introduced directly into CNS. Protection mediated via IFN-γ is presumably due to the activation of effector cells already present at the site of infection or recruited to the site, because IFN-γ activates macrophages to better kill cryptococci.[19] In addition, cryptococcal growth inhibition in the brain is probably due to the production of IFN-γ, which activates macrophages recruited to the CNS or endogenous effector cells, such as microglial cells or astrocytes, to kill the cryptococci.[20]

In vitro experiments with primary human microglia after exposure to *C. neoformans*, in the absence of specific antibody, showed that chemokine levels were not affected. However, in the presence of specific antibody, microglia-produced levels of MIP-1α and MIP-1β are in amounts comparable to those induced by lipopolysaccharide. In addition to MIP-1α and MIP-1β mRNA levels, a robust induction happened to MCP-1 and IL-8 mRNA levels following incubation of microglia with opsonized *C. neoformans*. In contrast, cryptococcal polysaccharide did not induce a chemokine response even when specific antibody was present and inhibited the MIP-1α induction associated with antibody-mediated phagocytosis of *C. neoformans*.

Treatment of microglia with cytochalasin D could inhibit internalization of *C. neoformans* but did not affect MIP-1α induction. In the opposite, treatment with a tyrosine kinase inhibitor, herbimycin A, could inhibit MIP-1α induction. Microglia stimulated with immobilized murine immunoglobulin could also produce MIP-1α. In summary, microglia can release several chemokines when stimulated by *C. neoformans* in the presence of specific antibody and this process is likely to be mediated by Fc receptor activation. This response can be down-regulated by cryptococcal capsular polysaccharide. These findings suggest a hypothetical mechanism, which can explain that *C. neoformans* infections fail to induce strong inflammatory responses in patients with cryptococcal meningoencephalitis.[21]

In an analysis of *C. neoformans* gene expression during experimental cryptococcal meningitis, the results indicated that the *C. neoformans* cells were actively engaged in protein synthesis, protein degradation, stress response, small molecule transport and signaling. In addition, a high level of energy requirement of the fungal cells was suggested by a large number of tags that matched putative genes for energy production. Taken together, these findings provide an insight into

the transcriptional adaptation of *C. neoformans* to the host environment and identify the set of fungal genes most highly expressed during cerebrospinal fluid infection.[22]

The Role of Toll Like Receptors (TLRs) in CNS Fungal Infections

The data about the role of TLRs, cellular receptors that mediate cellular responses to pathogen-associated molecular patterns (PAMPs), in CNS fungal infections are very limited. It is known that signalling by *Candida albicans* and *Aspergillus fumigatus* essentially occurs through TLR2, TLR4 and TLR9 that are implicated in different ways in the control of fungal infections. In addition, *Aspergillus* hyphae, unlike conidia and *Candida* hyphae, unlike yeasts, seem to be sensed through TLR4, which indicates that TLRs discriminate between distinct fungal morphotypes.[23]

In a murine model, stimulation of quiescent microglia with various TLR agonists including LPS (TLR4), peptidoglycan (TLR2) and CpG DNA (TLR9), activated the cells to up-regulate unique patterns of innate and effector immune cytokines and chemokines at the mRNA and protein levels.[24] Thus, microglia appear to be a very important component of both the innate and adaptive immune response, providing the CNS with a means to rapidly and efficiently respond to a wide variety of pathogens including fungi. However, TLRs and fungal infections of the CNS constitute a scientific area in which further investigation is warranted.

The Role of Complement System in CNS Fungal Infections

The complement (C) system is a key component of the innate immune system, playing a central role in host defense against pathogens.[25] It is also a powerful drive to initiate inflammation and can, if unregulated, cause pathology leading to severe tissue damage. However, only few reports are available concerning the role of C in fungal infections of the brain. Although commonly considered as non-pathogenic, *Saccharomyces cerevisiae* is accounted as the main agent of a number of cases of sepsis in patients with predisposing immune factors. It is important that the mean experimental fungal burden in the brain was several-fold higher in C5-deficient mice than in wild type control mice. In addition, mice lacking C5 had a significantly greater brain fungal burden after infection with *C. albicans*. Again, it is generally accepted that the C system is essential to mediate cytolysis of the fungi and furthermore, it is well known that C receptors expressed by activated microglia are important to mediate phagocytosis, for example of *Cryptococcus*.[26]

Summary

Although the data about immunopathogenesis of CNS fungal infections in humans are very limited, animal data can possibly explain some of the mechanisms and interactions taking part between brain cells and fungi during infection. The activation of brain resident cells combined with relative expression of immune-enhancing and immune-suppressing cytokines and chemokines may play a determinant role in immunopathogenesis. In addition, the exploration of the genomic sequence of most fungal pathogens can help better understand pathogenesis, virulence and immune response of host defense against these pathogens.

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