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Molecular mechanisms of cryptococcal meningitis

Tong-Bao Liu,[1] David S. Perlin[1,2] and Chaoyang Xue[1,2,*]

[1]The Public Health Research Institute; New Jersey Medical School; University of Medicine and Dentistry of New Jersey; Newark, NJ USA;
[2]Department of Microbiology and Molecular Genetics; New Jersey Medical School; University of Medicine and Dentistry of New Jersey; Newark, NJ USA

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Fungal meningitis is a serious disease caused by a fungal infection of the central nervous system (CNS) mostly in individuals with immune system deficiencies. Fungal meningitis is often fatal without proper treatment, and the mortality rate remains unacceptably high even with antifungal drug interventions. Currently, cryptococcal meningitis is the most common fungal meningitis in HIV-1/AIDS, and its disease mechanism has been extensively studied. The key steps for fungi to infect brain and cause meningitis after establishment of local infection are the dissemination of fungal cells to the bloodstream and invasion through the blood brain barrier to reach the CNS. In this review, we use cryptococcal CNS infection as an example to describe the current molecular understanding of fungal meningitis, including the establishment of the infection, dissemination, and brain invasion. Host and microbial factors that contribute to these infection steps are also discussed.

General Causes of Meningitis

Meningitis refers to inflammation of the meninges (membranes that cover the brain and the spinal cord), which is commonly caused by infection with pathogens, including bacteria, fungi, viruses or parasites. Despite advances in antimicrobial and antiviral therapy, meningitis still results in significant morbidity and mortality.[1] The most common symptoms of meningitis are headache, neck stiffness and pain, with associated fever, vomiting, seizures, confusion or altered consciousness and an inability to tolerate light or loud noises.[2]

The key step for a pathogen to infect brain and cause meningitis is to cross the blood brain barrier (BBB), an interface that separates the peripheral circulation and the central nervous system (CNS). The BBB occurs along all capillaries in the CNS and consists of tight junctions around the capillaries to regulate the passage of blood-borne substances into the brain and maintain the homeostasis of the neural microenvironment that is crucial for normal neuronal function (**Fig. 1**). This specialized system restricts microbe and large molecules in blood from entry into the brain, while allowing the diffusion of small hydrophobic molecules such as O_2, hormones and CO_2.[3,4]

Only limited numbers of pathogens are capable of invading the brain to cause meningitis. To successfully infect the brain, pathogens have evolved various strategies to penetrate the BBB. Pathogens may cross the BBB transcellularly, paracellularly and/or by the so-called "Trojan horse" mechanism.[5] Transcellular traversal refers to microbial penetration through human brain microvascular endothelial cells (HBMECs) without disrupting intercellular tight junctions. Paracellular traversal is defined as microbial penetration between barrier cells with and/or without disruption of tight junctions. This mechanism involves loosening of the tight junction or disrupting of its supporting components, such as glial cells and basement membrane.[6] The "Trojan horse" mechanism involves microbial penetration of the barrier cells using transmigration within infected phagocytes[4] (**Fig. 1**). All three mechanisms have been reported in bacterial pathogens to gain entry, while the "Trojan horse" mechanism is common in virus infection, such as HIV-1 and West Nile virus.[4]

A number of fungi have been shown to cause meningitis in humans, including yeast pathogens (*Cryptococcus neoformans* and *Candida albicans*), dimorphic fungi (*Histoplasma capsulatum*, *Coccidioides immitis, Paracoccidioides brasiliensis* and *Blastomyces dermatitidis*),[7] filamentous fungi (*Aspergillus* species and Zygomycetes) and several dematiaceous molds (*Bipolaris spicifera*, *Exophiala jeanselmei*, *Cladophialophora bantiana*, *Ochroconis gallopavum* and *Ramichloridium mackenziei*)[8-10] (**Table 1**). *Cryptococcus neoformans* is the causative agent for the most common fungal meningitis, especially in areas where HIV-1 is prevalent. Cryptococcal meningitis is uniformly fatal without proper treatment.[11,12] It is also the most extensively studied type of fungal meningitis. In this review, we use cryptococcal meningitis as a model to describe the current understanding of fungal meningitis.

Development of Cryptococcosis

Significance of cryptococcal meningitis. *C. neoformans* causes the most common fungal infection of the central nervous system (CNS) in HIV-1/AIDS populations with high mortality and morbidity. CNS cryptococcosis may present as encephalitis, meningitis or cerebral-space-occupying lesions. It is reported that cryptococcal meningitis occurs in 8% of patients with HIV/AIDS in the US and as much as 40% of these patients in other part of the world.[13] A recent epidemiologic analysis projected that there are around one million cases of cryptococcal meningitis in AIDS patients each year that are responsible for over 600,000 annual deaths.[12]

Environmental niches. There are two principal *Cryptococcus* species that often cause human and animal infections, *C. neoformans* (serotype A and D) and its sibling species *C. gattii*

*Correspondence to: Chaoyang Xue; Email: xuech@umdnj.edu
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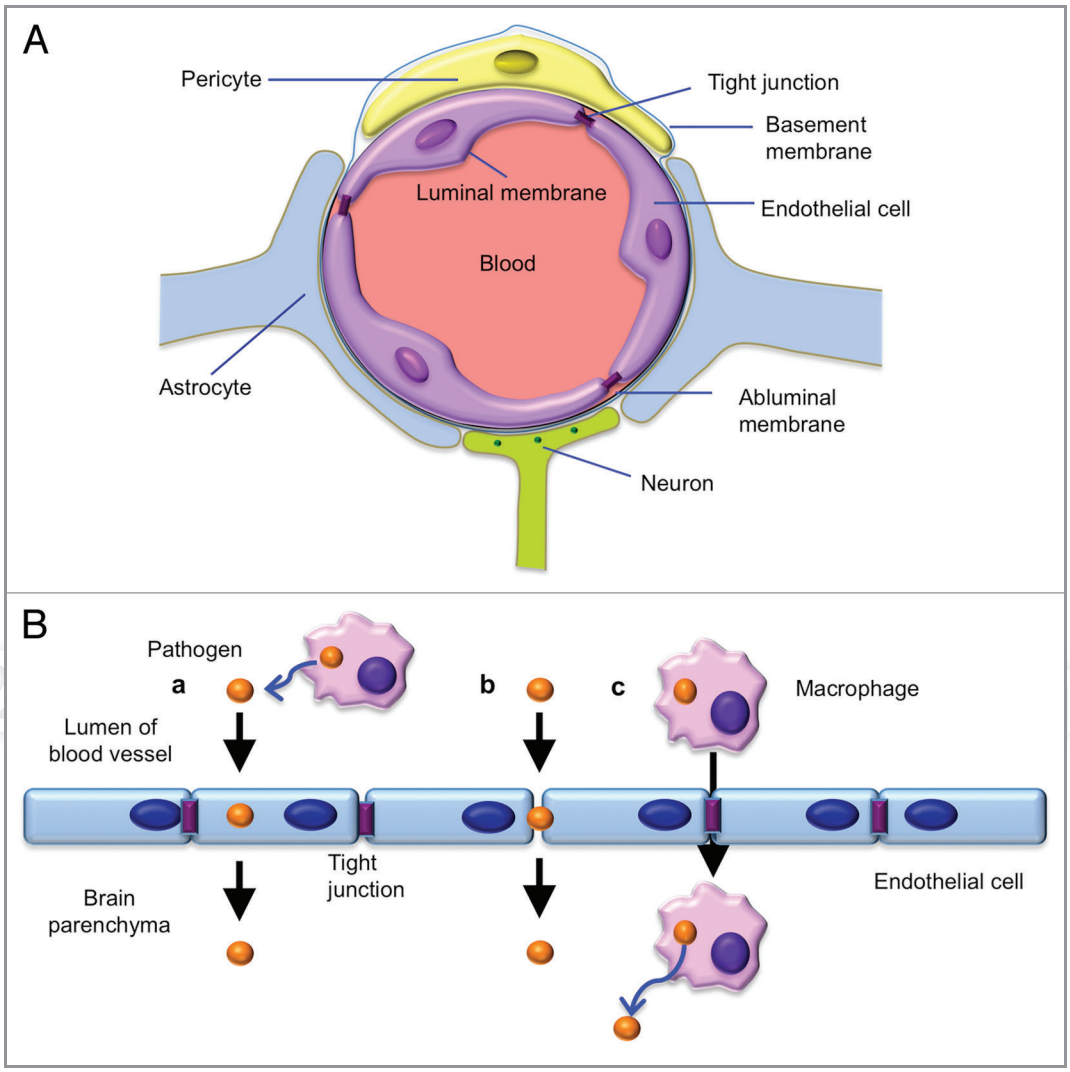

Figure 1. (A) The illustration of the blood-brain barrier (BBB). The BBB is a multi-cellular structure at the interface of circulation and the central nervous system. It is composed of brain microvascular endothelial cells, astrocytes, pericytes and neurons. The main function of the BBB is to maintain the neural microenvironment by regulating the changes of the levels of molecules in the blood, and protect the brain by blocking the entry of toxins and microorganisms that are circulating in the blood. (B) Pathogens can cross the BBB transcellularly, paracellularly and/or in infected phagocytes (the Trojan horse mechanism). In the transcellular traversal model (a), pathogens across the barrier by direct endocytosis of brain microvascular endothelial cells without disruption of intercellular tight junction. In the Paracellular traversal model (b), pathogens penetrate between barrier cells through loosen tight junction, and may or may not lead to tight-junction disruption. The "Trojan horse" mechanism (c) involves phagocytic microbial penetration of the barrier cells using transmigration within infected phagocytes. Pathogen cells are released from macrophages after penetration.

(serotype B and C). Cryptococcus exists ubiquitously in the environment with worldwide distribution. It was first isolated from peach juice samples in 1894.[14] There are several major environmental niches where Cryptococcus cells can be most frequently isolated. *C. neoformans* is commonly associated with soil and bird droppings and has a global distribution, whereas its sibling species *C. gattii* is traditionally a tropical and subtropical organism, associating with several tree species, including *Eucalyptus* spp.[15-18]

The detailed mechanism as to why Cryptococcus prefers these particular niches remains unclear. Our recent studies showed that inositol from plants plays an important role in stimulating sexual reproduction in *Cryptococcus* species, suggesting that Cryptococcus can utilize certain compounds from niches for its development, which may have broad implication of the host-pathogen co-evolution.[19] The observation that the sexual reproduction of *C. neoformans* occurs in media made of pigeon guano also suggests the benefit of environmental niches for the development of this microbe.[20] The recent outbreak of *C. gattii* infection in immunocompetent individuals on Vancouver Island, Canada and its expansion in Canada and the Pacific Northwest suggests an evolution of host range, geographic location, and virulence of this pathogen. This further underlines the complexity of its epidemiology and disease mechanism.[21-25]

Pulmonary infection. Cryptococcus spores, produced as a result of sexual reproduction, or desiccated yeasts are believed to

Table 1. Causative agents of fungal meningitis

Agents	Frequency of causing meningitis	Morphology	Disease geographic locations	Mechanism of BBB crossing	References
Cryptococcus neoformans	Most common	Yeast	Global	Transcellularly, "Trojan horse" model	10, 11, 13, 41, 75, 77, 112
Candida albicans	Less common	Yeast, dimorphic	Global	Transcellularly	9, 10, 112, 116
Histoplasma capsulatum	Common	Dimorphic	Ohio River and Mississippi River valleys, South America, South Asia, Sub-Saharan Africa	Unclear	10, 112
Coccidioides immitis	Common	Dimorphic	Southwest United States, South America	Unclear	10, 112
Blastomyces dermatitidis	Rare	Dimorphic	Midwest and Northern United States and Canada	Unclear	7, 10, 112
Paracoccidioides brasiliensis	Rare	Dimorphic	Unclear	Unclear	10, 112
Sporothrix schenckii	Rare	Dimorphic	Peru	Unclear	10, 112
Aspergillus spp	Rare	Filamentous	Global	Unclear	10, 112, 113
Zygomycetes	Rare	Filamentous	Unclear	Unclear	8, 10, 112
Exophiala jeanselmei	Rare	Dematiaceous molds	East Asia	Unclear	8, 10, 112, 115
Cladophialophora bantiana	Rare	Dematiaceous molds	Global	Unclear	8, 10, 112, 115
Ramichloridium mackenziei	Rare	Dematiaceous molds	Middle East	Unclear	8, 10, 112, 115

be the initial infectious particles in nature, which has been supported by in vivo studies using animal models.[26-28] Spores inhaled by human or animal hosts will lodge into lung alveoli. Cryptococcus can colonize the host respiratory tract without producing significant symptomatic disease, thus initial infection can be in a dormant or latent form. When host immunity is compromised, the dormant form may reactivate and disseminate hematogenously to cause systemic infection.[29] Cryptococcal exposure is prevalent, which was evident by a survey that indicates almost all adults in New York have antibody reactive to *C. neoformans,* an indication of exposure to this organism.[30]

For the host, containment of fungus in the lung is accomplished with a combination of cell-mediated immunity, innate immunity, as well as antibody responses.[31] Macrophages are the first line of host defense and complement-mediated phagocytosis is likely to be the primary initial defense against cryptococcal infection.[32,33] Other host factors important for defense against infection include $CD4^+$ and $CD8^+$ T cells, as well as cytokines such as tumor necrosis factor (TNF)-α, interferon (IFN)-γ, and interleukin (IL)-18.[34,35] Antibodies are part of the immune response to cryptococcal infection, as animal studies have shown that cryptococcal infection treated with antibodies against the capsule component glucuronoxylomannan (GXM) can reduce the fungal burden and enhance animal survival.[36] If the host immune system fails to contain the fungus, Cryptococcus can infect and spread to other organs to cause infections involving almost any part of the body, including the skin, eyes, myocardium, bones, joints, lungs, prostate gland, or urinary tract, as well as the central nervous system (CNS).[33]

Dissemination. Dissemination occurs when the host defense mechanism fails, i.e., when phagocytic cells fail to kill the yeast, instead serving as a niche for fungal replication. Fungal cells can be disseminated by moving through macrophages or other mechanisms to reach the blood circulation. Yeast cells can replicate inside macrophages to form cryptococcal phagosomes, which then lead to the burst of host macrophages to release fungal cells. Cryptococcal cells can also exit macrophages by extrusion without lysis, allowing both the host cell and pathogen to survive.[37-39] A recent report showed that a WASP-Arp2/3 complex-mediated actin polymerization mechanism is involved in temporarily inhibiting expulsion of yeast cells from infected phagocytes.[40] Excited yeast cells can then be immediately internalized by another macrophage without encountering the extracellular environment to avoid the host defense system.[37-39]

Cryptococcal cells are rapidly cleared from the bloodstream by the host defense system. Studies using a murine model showed that only 1.7% to 2% of yeast cells deposited in the bloodstream through tail vein injection survived after 30 min.[41] One potential mechanism for yeast cells to survive and disseminate is to maintain and replicate inside macrophages, as macrophages can be utilized as a niche for replication if they fail to kill yeast cells.[37,39] Yeast cells reach the BBB through the blood circulation and cross it to reach the meninges, where rapid replication occurs to cause inflammation and subsequent meningoencephalitis. Alternatively, macrophages containing yeast cells may be able to directly cross the BBB to deliver fungal cells inside the brain.

Both cryptococcal pneumonia and cryptococcal meningitis are serious disease manifestations and are potentially fatal. There is no clear evidence of human-to-human transmission, thus human and animals are likely the terminal host, and cells can only be recycled in nature from demise hosts.

Pathogen factors involved in the cryptococcal pulmonary infection and dissemination. There are several well-characterized virulence factors that contribute to the success of a fungal infection. Tolerance to mammalian body temperature 37°C is a precondition for the establishment of infection. The thermal

tolerance of *C. neoformans* is linked to cell integrity and is controlled by multiple primary signaling pathways, including the calcineurin pathway[42] and the protein kinase C1 (PKC1)-activated MAP kinase (Mpk1) pathway.[43,44] Cryptococcal cells are covered by a large polysaccharide capsule, which is induced by CO_2,[45,46] phospholipids[47] and low iron conditions.[48] Capsule formation protects cells from phagocytosis.[33,49] Its capsular polysaccharide glucuronoxylomannan (GXM) component interferes with T-cell function, disrupting cell-mediated immunity.[50-53] The polysaccharide capsule is also an important microbial factor affecting dissemination. Following intravenous injection in a murine model, around 0.2% CFU of an encapsulated strain was recovered in the circulating blood after 24 h of inoculation, while no yeast cells of an acapsular strain were detected after 2 h following the infection, indicating the importance of capsule in dissemination.[41] This finding is consistent with the role of GXM in inhibiting T-cell activation and proliferation, thus preventing cryptococcal cells from being cleared in the blood.[50,54] In addition, Cryptococcus contains a thick cell wall with the deposition of phenolic melanin, which has been proposed to protect cells from oxidation.[55,56] Laccase, a key enzyme required for melanin biosynthesis, is also important for fungal virulence.[57,58] Mutant strains lacking laccase production show virulence attenuation due to inability to escape from the lungs, without affecting growth in either the blood or the brain, indicating that laccase plays a role in the dissemination of Cryptococcus into the bloodstream.[58] Both capsule formation and melanin production are controlled by the Gpa1 G protein signaling pathway via regulation of cellular cAMP levels.[59,60]

Enzymes involved in inositol metabolism pathways have been found to be important for pathogenicity in *C. neoformans*.[61,62] Inositol sphingolipid biosynthesis and breakdown are required for Cryptococcus pathogenicity by regulating intracellular and extracellular fungal growth.[61,63-65] The inositol phosphoryl ceramide synthase 1 (Ipc1) and its downstream protein App1 (antiphagocytic protein 1) were both found to be important for regulating Cryptococcus phagocytosis and potential dissemination.[61,66] Meanwhile, cryptococcal inositol phosphosphingolipid phospholipase C1 (Isc1), an enzyme that breaks down inositol sphingolipids, has been shown to be important for the survival of *C. neoformans* in activated macrophages.[67] The *isc1*Δ mutant strain produces much less fungal burden in the lung when compared with the wild type strain in a murine model, and fails to disseminate due to its inability to survive after phagocytosis by activated macrophages.

The secreted enzyme phospholipase B1 (Plb1) is also important for the dissemination of Cryptococcus into the bloodstream. Plb1 is a multifunctional enzyme containing activities of phospholipase B, lysophospholipase and lysophospholipase transacylase.[68,69] Compared with wild-type, mice infected by *plb1Δ* mutant strains show a reduced fungal burden in lung interstitial macrophages and extrapulmonary sites, including brain, suggesting Plb1 is required for both the initiation of infection and dissemination.[70]

Different fungal mating types have also been demonstrated to have different outcomes for disease progression. When animals are co-infected with mixtures of α and **a** mating type cells, α cells preferentially disseminate to the CNS.[71] Recent studies demonstrate that the pheromone sensing during co-infection in vivo leads to the production of much enlarged **a** cells (giant cells/titan cells) that prevent them from effectively undergoing phagocytosis.[71,72] The size of titan cells could be as large as 100 uM in diameter, 10x larger than typical cryptococcal cells. Because survival in macrophages is important for fungal cell trafficking and dissemination, such morphological transition causes a defect in **a** cell dissemination from lung to the bloodstream.[72,73] G protein-coupled receptors (GPCRs) Ste3**a** and Gpr5 have been shown to regulate the gigantism of cryptococcal cells via the activation of G protein Gpa1 and its downstream signaling pathway (Gpa1-Pka1-Rim101 signaling cascade).[74] Meanwhile, phospholipids have been reported to stimulate the giant cell formation.[47] It would be interesting to investigate whether certain GPCRs, such as Ste5, can sense phospholipid as a ligand.

Mechanism of Brain Infection

Penetration of the BBB is the key step for pathogens infecting brain to cause meningitis. Fungal cells are much larger in size compared with viruses or bacteria that often cause meningitis. How fungal cells cross the BBB to invade brain is a fundamental question for studying fungal meningitis. Although this key issue is not completely understood in cryptococcosis, recent research advances have suggested that multiple mechanisms likely exist. Both a direct transcellular transmigration mechanism and the "Trojan horse" model have been reported in Cryptococcus.[41,75-77]

Transcellular model. The transcellular model was supported by results from experimental mouse models of cryptococcal meningitis following intravenous inoculation, as well as cases of human cryptococcal meningitis.[41,76,78] *C. neoformans* invasion into the brain following fungemia was reported to occur via cell entry through cerebral capillaries, not the choroid plexus.[79] It can enter and traverse human brain microvascular endothelial cells (HBMECs) without any obvious change in HBMEC integrity. Observations with transmission and scanning electron microscopy have revealed that *C. neoformans* induces the reorganization of host cell cytoskeletal structures and the formation of microvilli-like protrusions to initiate its entry into HBMECs.[41,80] *C. neoformans* is found intracellularly in membrane-bound vacuoles and no free cryptococcal cells are found in the HBMEC cytoplasm. Once internalized, *C. neoformans* causes minimal endothelial cell damage and exits from the surface of the cell.[41,81] These findings indicate that *C. neoformans* can use a transcellular mechanism to enter HBMECs that involves host cell actin cytoskeleton rearrangements. Further studies revealed that the invasion is mediated through the lipid rafts-endocytic pathway that involves the molecule ganglioside GM1, cell surface glycoprotein CD44 protein, cytoskeleton and intracellular kinase-DYRK3 (dual specificity tyrosine-phosphorylation-regulated kinase 3).[82,83] Hyaluronic acid was identified as the fungal ligand that directly interacts with CD44 from lipid rafts during Cryptococcus adhesion and invasion of HBMEC. Also, the host cell protein kinase C α isoform has been reported to be important

for the BBB crossing by regulating actin filament activity.[84] HBMEC treated with either PKC inhibitors or F-filament-disrupting agents inhibits fungal invasion into the endothelial monolayer.

A recent study using intravital microscopy to generate real-time imaging of cryptococcal transmigration in mouse brain elegantly show that both yeast cells and polystyrene microsphere with similar size stop moving suddenly once reach mouse brain capillaries of similar or smaller diameter than the particle, and then only viable cells can cross the capillary wall. These results indicate that cryptococcal cells are mechanically trapped in the brain capillary during dissemination but actively transmigrate to the brain parenchyma. This observation is in agreement with the transcellular model.[75]

It has been suggested that a potential paracellular transmigration mechanism might exist in *C. neoformans*. A major tight junction maker protein, occludin, was found rapidly degraded in connection to the actin cytoskeletal rearrangement in endothelial cells during the Cryptococcus-HBMEC interaction, an indication that Cryptococcus might have a profound impact on the integrity of HBMEC tight junction. A loosen or disrupted tight junction might allow yeast cells to pass.[80] However, a direct evidence for such a mechanism is still lacking.

"Trojan horse" model. The importance of macrophages in the cryptococcal infectious cycle has been well-documented.[85,86] Cryptococcus is a facultative intracellular pathogen that can survive in macrophages as a niche for replication and for avoiding the hostile host environment.[87] Yeast cells can escape alive from phagocytic cells by an active mechanism of phagosomal extrusion and then invasion of other phagocytes.[37-39] The extrapulmonary dissemination is macrophage associated, suggesting a tight association between yeast cells and macrophages during infection.[67,88] Cryptococcal cells were also observed to be closely associated with phagocytic cells in the meningeal vasculature,[76] which supports the hypothesis of the "Trojan horse" model. The direct evidence for a "Trojan horse" model came from a recent report where mice were infected with macrophages containing ingested cryptococcal cells.[77] In this report, bone marrow-derived monocytes (BMDM) infected with Cryptococcus were used for mouse intravenous infection. Compared with mice infected by free yeast cells, a 4-fold higher brain CFU was observed for the BMDM yeast. Late phagocyte depletion obtained by clodronate injection reduced disease severity and lowered the fungal burden by 40% in all organs studied. These results provide evidence for "Trojan horse" crossing of the BBB by *C. neoformans* and overall for a role of phagocytes in fungal dissemination.[77]

Virulence factors important for brain infection. Studies have shown that various cryptococcal virulence factors contribute to extrapulmonary dissemination, including the capsular polysaccharide, mannitol, the mating type, melanin, phenotypic switching, phospholipase, prostaglandins and urease.[11] Several virulence factors have also been identified to play an important role in the transversal of Cryptococcus across the BBB and brain infection. Fungal cell morphology and capsule morphology have been reported to play a role in the BBB crossing. The production of giant/titan cells during infection prevents those cells from being engulfed by macrophages, thus cause ineffective dissemination from lung to the blood stream.[72] It is reasonable to speculate that the enlarged cells also could be more difficult to invade the BBB simply due to their size. The variation of Cryptococcus cell morphology referred as smooth, mucoid, or wrinkled form has been described.[89] The smooth *C. gattii* cells were reported to produce smaller capsules in general and are more efficient in crossing the BBB and causing CNS infection.[90]

Cryptococcal urease has been found to contribute to dissemination to the central nervous system (CNS) following intravenous inoculation, but not the growth in the CNS.[78] Further study using real-time imaging microscopy showed that urease does not affect the trapping of the yeast in the capillary or the replication in the brain, but plays a role in the transmigration of yeast cells.[75] It was proposed that the product of urease, ammonia, might introduce a local damage of the endothelium, thus increasing permeability and leading to transmigration of Cryptococcus.[75]

Fungal inositol transporters have also been found to play a role in cryptococcal virulence, including brain infection.[19,91,92] Several factors point to inositol as a potential host factor promoting the development of cryptococcal meningitis. First, both human and animal brains contain abundant free inositol, which plays a critical role in the normality of neurological response and psychological feedbacks.[93,94] Inositol is a major osmolyte in the human and animal brains. It is present in the human cerebellum at over 200-fold higher concentrations than found in plasma.[93] Second, Cryptococcus can utilize inositol as a sole carbon source, which may provide a growth advantage during brain infection.[95,96] Evidence from pathological studies showed that Cryptococcus cells associate with inositol-rich microglial cells during CNS cryptococcosis.[97,98] Third, an unusually large inositol transporter gene family with over ten members has been identified in Cryptococcus, and they appear to play an important role in fungal virulence.[19,91,92] Mutants lacking two major fungal inositol transporters Itr1a and Itr3c showed attenuated virulence in several murine models (intranasal inhalation model, intravenous injection model and intracerebral injection model), indicating the fungal inositol acquisition system is required for Cryptococcus-host interaction, particularly during brain infection. Recently, we found that inositol can directly increase the rate of Cryptococcus transversal across the BBB in an in vitro BBB model, and the inositol effect is fungal inositol transporter-dependent (our unpublished results). This discovery suggests that inositol sensing and utilization could be another important virulence factor for development of cryptococcal meningitis.

The cryptococcal inositol phosphosphingolipid phospholipase C1 gene (Isc1) has also been shown to be important for controlling the dissemination of *C. neoformans* to the brain in mice. The *isc1Δ* mutant produces hyperencapsulated cells and fails to invade brain to cause CNS cryptococcosis.[67] This also suggests that capsule size is important for fungal dissemination to the CNS and the development of *C. neoformans* meningoencephalitis. Phosphatidylinositol 4-kinase (Pik1), an enzyme involved in inositol metabolism to produce phosphatidylinositol 4-phosphate, has been reported to be essential for fungal survival on medium

containing cerebrospinal fluid (CSF) in vitro and during brain infection in a rabbit meningitis model in vivo.[99]

The Cryptococcus Plb1 has been reported to be secreted in a phosphatidylinositol transfer protein Sec14-dependent manner.[100] Both in vitro and in vivo experiments have established that Plb1 facilitates adherence to lung epithelium, establishment of interstitial lung infection, survival and replication of Cryptococcus within macrophages, and dissemination to the CNS.[70,101,102] A defect in dissemination of *plb1*Δ mutants may be due to the reduced ability for yeast cells to escape from macrophages, a process commonly called vomocytosis.[100]

Lastly, a number of regulatory factors, including the sterol regulatory element-binding protein (Sre1)[103] and copper-dependent transcription factor 1 (Cuf1),[104] contribute to the infection of *C. neoformans* in the brain. Mice infected by the *sre1*Δ mutant showed an accumulation of yeast cells in the meningeal layer but no cystic lesion development in the brain, suggesting that Sre1 is important for adaptation and growth in the brain but is dispensable for invasion into the brain.[103] Meanwhile, Cuf1 was found to play a role in the dissemination of cryptococcal cells to the brain, but not their proliferation in the lung based on mouse models. Cuf1 regulates the expression of the low affinity copper transporter Ctr4 in *C. neoformans*. The expression level of *CTR4* is upregulated during brain infection and its expression pattern in patients with systemic cryptococcosis is highly correlated with Cryptococcus growth in the brain.[104] These observations suggest that copper acquisition is important for fungal pathogenesis during neurologic infection.

Animal Models for Cryptococcal Meningitis

Besides causing human infection, Cryptococcus is also a wide-range veterinary pathogen that naturally infects cats, dogs, cows, horses and primates, as well as some invertebrate species. The similarity of disease infections and clinical manifestation between human and animals has led to the development of several excellent animal models for cryptococcosis, including rabbit, mouse, guinea pig and rat.[105] The guinea pig was the first animal model system selected for the study of cryptococcosis,[106] and the model has been improved for antifungal therapy study. Its medium body size makes it a good chronic infection model for antifungal therapy studies.[107] The disadvantage is that the nature of the immune response is not well studied, and only a few inbred guinea pig strains exist. Rabbits are naturally resistant to cryptococcal infection, and immune suppression with corticosteroids is commonly used to circumvent intrinsic host resistance. The strong correlation between rabbit response to antifungal treatment and human disease outcome makes the rabbit a valuable model host for cryptococcal meningitis.[108,109] The large size of rabbits allows for frequent, repetitive sampling of CSF, facilitating the investigation of chronic cryptococcal meningitis.[109] A potential limitation of the rabbit model is the availability of only a few inbred strains, few molecular tools and the requirement for immune suppression. The mouse is the most popular model host for laboratory study of cryptococcal infection. The mouse intranasal inhalation model mimics the natural route of cryptococcal infection. The mouse intravenous injection model directly deposits fungal cells in the blood circulation and is commonly used for testing the BBB crossing, while the mouse intracerebral injection model is used for studying the development of CNS cryptococcosis. The major advantage of this model is its relatively low cost, ease of handling, the availability of numerous inbred strains with well-developed tools for immunologic and genetic investigation and the ability to produce a clinically relevant experimental cryptococcal infection.[110] Rats have features common to mice, but its relative large size is advantageous for the experimental procedures such as intratracheal infection or sampling of CSF.[111]

Meningitis Caused by Other Fungi

Besides cryptococcal meningitis, several other medically important fungi can also infect brain to cause meningitis with high mortality. Most fungi causing CNS infection are saprobes with worldwide distribution; a few are geographically restricted like *Coccidioides immitis*.[112] Among them, CNS infection by the dimorphic fungi *Histoplasma capsulatum* or *C. immitis* is well described but an uncommon manifestation of disseminated disease. Other medically important fungi, including *Aspergillus* spp,[113] *Candida* spp, *Blastomyces dermatitidis*, *Paracoccidioides brasiliensis*, as well as Zygomycetes can also infrequently cause meningitis[10] (**Table 1**). In addition, a subgroup of the dematiaceous molds (Phaeohyphomycoses), such as *Bipolaris spicifera*, *Exophiala jeanselmei*, *Cladophialophora bantiana*, *Ochroconis gallopavum* and *Ramichloridium mackenziei*, also have been reported to cause often lethal CNS infection even in immunocompetent patients.[8,114,115] The fact that these dematiaceous molds and Cryptococcus both produce dark brown melanin and both have CNS tropism is an interesting correlation. It would be interesting to investigate whether there is any commonality in the molecular mechanism of brain invasion among these fungi.

Compared with cryptococcal meningitis, mechanistic understanding of the disease development in other fungal meningitis remains limited. It has been reported that the transcellular mechanism is used for transversal of *Candida* spp across the BBB.[116] Candida invasion of brain endothelial cells has been shown to be mediated by the fungal invasins, Als3 and Ssa1, through interacting with host cell receptor gp96.[117] *H. capsulatum* cell surface protein Yps3 has been reported to play a role in fungal transition and pathogenicity.[118] This protein has also been found to induce Toll-like receptor 2 (TLR2) signaling, including the activation of NFκB in microglial cells, indicating that Yps3 plays an important role in Histoplasma CNS infection.[119] Very few experimental data have been established on how other fungi invade brain. Meanwhile, current strategies on disease epidemiology, diagnosis, and antifungal therapy of fungal meningitis caused by these fungi have been well documented in a number of excellent reviews.[9,10,112,115,120]

Concluding Remarks and Future Perspective

Despite awareness of HIV/AIDS and the application of widespread antiretroviral therapy that reduced overall opportunistic

infections in AIDS, the mortality and morbidity rate caused by fungal meningitis remains unacceptably high, especially in resource-limited certain regions of the world. Also, the advance of modern medical interventions, such as cancer chemotherapy, organ transplantation, and other therapies, have increased immunocompromised populations without HIV-1 infection that are also susceptible to opportunistic fungal infections. Fungal meningitis remains a serious medical problem even in the countries with advanced healthcare systems. Molecular studies on host-pathogen interactions during systemic fungal infections remain an urgent priority to better understand disease mechanisms that will help formulate better disease control strategies.

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