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Host Immune Responses in the Central Nervous System during Fungal Infections

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SUMMARY

Fungal infections in the central nervous system (CNS) cause high morbidity and mortality. The frequency of CNS mycosis has increased over the last two decades as more individuals go through immunocompromised conditions for various reasons. Nevertheless, options for clinical interventions for CNS mycosis are still limited. Thus, there is an urgent need to understand the host-pathogen interaction mechanisms in CNS mycosis for developing novel treatments. Although the CNS has been regarded as an immune-privileged site, recent studies demonstrate the critical involvement of immune responses elicited by CNS-resident and CNS-infiltrated cells during fungal infections. In this review, we discuss mechanisms of fungal invasion in the CNS, fungal pathogen detection by CNS-resident cells (microglia, astrocytes, oligodendrocytes, neurons), roles of CNS-infiltrated leukocytes, and host immune responses. We consider that understanding host immune responses in the CNS is crucial for endeavors to develop treatments for CNS mycosis.

Keywords

Fungal; Toll-like Receptors/Pattern Recognition Receptors; Neuroimmunology; microglia; astrocytes; neurons

1 | INTRODUCTION

Incidences of invasive opportunistic fungal infections have risen dramatically over the last two decades and are expected to continuously rise because of increasing numbers of individuals with impaired immunity due to HIV/AIDS, genetic disorders, aging, and immunosuppressive therapies. Accordingly, clinical cases of fungal infections in the CNS are also increasing. CNS mycoses are particularly severe due to limited treatment options, causing high morbidity and mortality. Over 5 million fungal species are estimated to exist, and about 70,000 fungal species have been described[1]. Of these, only a few hundred are known to cause disease in humans[2]. A small number of fungi are known to have

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CONFLICTS OF INTEREST
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neurotropism, and such fungal genera include but are not limited to *Cryptococcus, Candida, Aspergillus, Mucor, Rhizopus, Blastomyces, Coccidioides,* and *Histoplasma*. Even with this relatively limited variety of known fungal pathogens, CNS mycosis is difficult to treat and causes devastating outcomes to patients.

When fungal clearance fails at the initial infection site, neurotropic fungi disseminate and enter the CNS through the bloodstream[3]. Although the blood-brain barrier (BBB) restricts the entrance of fungi into the CNS parenchyma, fungi use various tactics to breach the barrier. The host response will then depend on CNS-resident cells to recognize the presence of fungi that have infiltrated through the BBB. Host CNS-resident cells indeed express a wide range of pattern-recognition receptors (PRRs). Yet, fungi also use stealthy approaches to evade host detection. Even when detection of invading fungi is successful, immune responses in the CNS require tight and masterful regulation to limit collateral damages to CNS-resident cells. Concomitantly, leukocytes from the circulation are also recruited to the infected CNS, further challenging the fine-tuning of host immune responses. This review intends to highlight the urgency of basic and mechanistic understanding of CNS immune response in the context of fungal infections.

2 | FUNGAL PATHOGENS THAT INFECT THE CNS

Candida, Cryptococcus, and *Aspergillus* are the three major fungal genera that typically cause fungal infections and disseminate to the CNS[3–6]. However, other fungi can also infect the CNS. These include non-Aspergillus molds, including fungi in the Mucorales order, and dimorphic fungi, such as *Blastomyces*, *Coccidioides,* and *Histoplasma*[3,6,7].

2.1 | *Candida* species

Candida is a genus of yeast fungi within the phylum Ascomycota and is the most common infectious fungi. Globally, it is estimated that there are ~750,000 cases of invasive candidiasis per year, with up to 40% mortality, depending on patients' predisposing factors[5]. *Candida* species can be harmless commensals located on mucosal surfaces, mainly in the gastrointestinal, respiratory, reproductive tracts, and the skin. Yet, they can be pathogenic when the mucosal or skin barriers are breached or compromised. *Candida* shows morphologies of yeast, pseudohyphae, or true hyphae during infection[8]. The majority of *Candida*-related CNS infections are caused by hematogenous spread of *Candida albicans* and cause meningoencephalitis[4]. In addition to disseminated *Candida* infection (candidemia), *Candida* CNS infections can be caused by neurosurgery of contaminated surgical devices[7]. Less commonly, *Candida* infection can cause chronic meningitis, brain abscesses, vasculitis with cerebral infarctions, spinal infections, ventriculitis, and mycotic aneurysms.

2.2 | *Cryptococcus* species

Cryptococcus is a genus of Basidiomycota fungi. They are yeast-like fungi but can also exhibit a hyphal state. However, they are not traditionally thought to be dimorphic since hyphal production typically occurs during the mating process. *Cryptococcus* yeasts are surrounded by a thick "capsule" consisting of polysaccharides, such as

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glucuronoxylomannan (GXM)[9]. The capsule enables *Cryptococcus* to evade immune recognition[10]. Currently, the global incidence of cryptococcal meningitis is about 223,100 cases per year and causes 15% of AIDS-related deaths globally[5,11]. *Cryptococcus neoformans*, an opportunistic fungus, is the most common species among the *Cryptococcus* genera. *C. gattii* is an endemic species, which infects immunocompetent individuals. An outbreak of *C. gattii* was reported two decades ago in the US Pacific Northwest[12]. Meningitis and meningoencephalitis are typical features of CNS cryptococcosis, most often caused by *C. neoformans*, and less commonly by *C. gattii*. CNS cryptococcosis often arises from a primary lung infection. *Cryptococcus* infection is a common complication of untreated HIV infection[13], which can cause immune reconstitution inflammatory syndrome (IRIS) upon immune reconstitution by HIV antiretroviral therapy[14,15].

2.3 | *Aspergillus* species

Aspergillus fungi belong to Ascomycota, like *Candida*, but *Aspergillus* are conidial fungi, which go through asexual reproduction by forming conidia. Because *Aspergillus* conidia are airborne; lung infection through inhalation is the most common etiology of the infection[16]. Invasive aspergillosis is severe and aggressive. It is estimated that as many as 10 million individuals are at risk for invasive aspergillosis globally, with more than 300,000 cases reported yearly with an associated mortality range of 30–80%[5,17]. *Aspergillus fumigatus* typically causes CNS infections through hematogenous spread from the primary site of infection, mainly the lung, or from a contiguous anatomical site such as the paranasal sinuses[4]. Although *Aspergillus* meningitis is relatively rare, cases are found more frequently in immunocompetent than immunocompromised patients[18].

2.4 | Mucorales order

Mucorales, including *Rhizopus* and *Mucor,* are mold fungi that belong to the phylum Mucormycota, considered to be phylogenetically more primitive than Basidiomycota and Ascomycota. Globally, the prevalence of mucormycosis is greater than 10,000 cases[5] with mortality rates of up to 95%[19]. The most common clinical presentation is rhino-orbital cerebral mucormycosis (ROCM), which are mainly caused by *Rhizopus oryzae*[20]. Although mucormycosis is relatively rare, immunocompromised individuals, as in hospital outbreaks, and diabetic patients are at high risk[20]. COVID-19-associated mucormycosis recently reported from India also raised a grave concern[21]. Routes of infection are inhalation, open wounds, and ingestion of contaminated food[22]. Cerebral mucormycosis is considered to be the most aggressive CNS fungal infection and uniformly fatal[7]. Nevertheless, how our immune system responds to Mucorales infections is still largely unknown.

2.5 | Other neurotropic fungi (including dimorphic fungi)

Despite being less common than the fungi described above, other fungal pathogens can infect the CNS. Depending on the growth conditions, they include dimorphic endemic fungi that grow as yeast or as mold[23]. The group of fungi, including *Blastomyces dermatitidis, Coccidioides* spp., and *Histoplasma capsulatum*, can cause meningoencephalitis, hydrocephalus, brain or epidural abscesses, and spinal cord lesions[6]. Other groups of fungi that could infect the CNS are molds other than *Aspergillus* and

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Mucorales (including *Fusarium*, *Scedosporium* species, Dematiaceous molds)[7] and yeast fungi other than *Candida* and *Cryptococcus* (including *Sporothrix*)[24].

3 | MECHANISMS OF CNS FUNGAL INVASION

Fungal infiltration into the CNS parenchyma predominantly occurs when host immune responses are inadequate in clearing pathogens at the primary infection site. To establish CNS infection, fungi need to penetrate the BBB, which consists of four different cell types: endothelial cells, pericytes, astrocytes, and microglia. The BBB exists at the interface of blood vessels and interstitial fluid in the brain and spinal cord, limiting microorganisms from entering the CNS. Pathogenic fungi secrete various molecules that induce host cell death, damaging host barriers. Fungi can also "hitchhike" host immune cells, *i.e.,* taking advantage of host cells as carriers, to invade the CNS.

In this section, we discuss mechanisms of fungal dissemination by the three major CNS-infecting fungal genera, *Cryptococcus*, *Candida*, and *Aspergillus*. There are three proposed models of fungal BBB penetration; transcellular migration (transcytosis), paracellular migration, and the Trojan Horse model. Reports have demonstrated that at least some species in *Cryptococcus*, *Candida*, and *Aspergillus* employ these dissemination methods to infiltrate the CNS.

3.1 | Transcellular migration (transcytosis)

Transcellular migration (termed transcytosis) is achieved by two different mechanisms. One is absorptive-mediated transcytosis (AMT), which does not require ligand-receptor binding but utilizes charge interactions[25]. The other mechanism is receptor-ligand mediated transcytosis (RMT), which requires specific binding between microbial ligand and a host cell receptor[25]. Transcytosis is not limited to CNS entry. Fungi take advantage of host cell transcytosis to exit the initial site of infection and cross the barriers to enter the bloodstream or lymphatics.

The transversal crossing of *C. neoformans* through brain microvascular endothelial cells (BMECs) is the essential step for developing cryptococcal meningoencephalitis (CM). Adhesion and transcytosis of cryptococci through human BMECs are observed, even with an acapusular mutant of *C. neoformans*[26]. As an example of RMT, hyaluronic acid (HA) on *C. neoformans* binds CD44 and 'receptor of hyaluronan-mediated motility' on human BMECs[27,28] (Fig. 1A). Indeed, CD44-deficient mice show a reduced fungal burden of *C. neoformans* in the brain[28]. For cryptococci to attach to the brain endothelium, *C. neoformans* also secretes a metalloprotease, Mpr1[29]. To assess the potency of Mpr1 in fungal dissemination, a study used *Saccharomyces cerevisiae*, which does not express Mpr1 and does not invade human BMECs[30]. Artificial expression of Mpr1 in *S. cerevisiae* successfully allowed the fungus to pass through the brain endothelium[29]. Because *S. cerevisiae* does not express HA, the result suggests that Mpr1 plays a critical role in transcytosis[29]. Another study showed that host Annexin A2 and its synergistic partner S100A10 mediate *C. neoformans* transcytosis[31]. In addition, *C. neoformans* releases microvesicles (termed "virulence bags"), containing polysaccharides, lipids, and cytoplasmic proteins[32]. The microvesicle contents fuse with host cells, as observed in human BMEC

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culture and *in vivo* mouse model of CNS infection[32]. Such microvesicles are also considered to be involved in the adhesion of *C. neoformans* to host BMECs. Furthermore, when *C. neoformans* crosses human BMEC monolayers, the host cell actin cytoskeleton is rearranged without affecting the integrity of the endothelial barrier[26,33]. Thus, without causing endothelial barrier disruption, *C. neoformans* can stealthily disseminate into the CNS without triggering host antifungal responses. Yet, a study suggested that *C. neoformans* ultimately induces endothelial cell necrosis[34].

C. albicans express fungal invasins to achieve brain invasion. One invasin is Als3, which binds a heat shock protein, gp96, expressed specifically on the surface of brain endothelial cells and promotes *Candida* uptake by the host cells[35]. Als3 also interacts with human cadherins to aid *Candida* endocytosis, as described in a study using human umbilical vein endothelial cells and human oral epithelial lines[36]. Epidermal growth factor receptor (EGFR) and human epidermal growth factor receptor 2 (HER2) cooperatively induce endocytosis of *C. albicans* in an Als3-dependent manner[37], which complements the cadherin-dependent mechanism for endocytosis. These studies suggested Als3 as a critical molecule for *Candida* transcytosis. Independent of the yeast-mediated mechanism, the hyphal form of *C. albicans* also contributes to the loss of BBB integrity in disseminated candidasis[38]. *C. albicans* can pass through the BBB by developing pseudo-hyphae, a virulence mechanism that does not elicit host cell morphological changes and enables the fungus to adhere, invade, and transcytose across human BMEC without affecting monolayer integrity[30].

Transcytosis of *A. fumigatus* across the BBB and infiltration into the CNS remains to be addressed. Yet, it is reported that sialic acid-specific lectin on *A. fumigatus* conidia adheres to fibrinogen or laminin on host cells[39]. Additionally, galactosaminogalactan, a cell wall-associated polysaccharide of *A. fumigatus*, has been identified as an adhesin[40]. Endocytosis of *A. fumigatus* conidia was identified in the A549 human lung epithelial cell line, human umbilical vein endothelial cells, and the J774 murine macrophage line[41]. Conidia internalization is promoted by fungal gliotoxin, which activates the actin cytoskeleton of host cells[42]. This mechanism is implicated in *A. fumigatus* transcytosis because gliotoxin alters the BBB integrity, at least *ex vivo,* without disrupting tight junction complexes[43]. Once *A. fumigatus* gains access to the brain, conidia germinate and establish CNS infection.

3.2 | Paracellular transport

In paracellular transport, microbes pass through barriers between epithelial cells by disrupting tight junctions without damaging host cells (Fig. 1B). Like transcytosis, paracellular CNS penetration also requires the attachment of fungi to the host cells before they are transferred.

C. neoformans employs paracellular transport[44,45], although paracellular transport is likely a minor route for migration across the BBB[34]. Ureases, cryptococcal virulence factors[46], compromises barrier cell tight junction integrity[47]. The approach is also used by *C. albicans*, which proteolytically degrades host E-cadherin to pass through epithelial cells, as reported in a study using oral epithelial cells[48]. To target E-cadherin, *C. neoformans* uses host IL-33, which is highly expressed during pulmonary cryptococcosis[49]. The study demonstrated that E-cadherin downregulation depends on IL-33[49].

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Electron microscopy captured that *A. fumigatus* conidia, which are not engulfed by host immune cells, can form extracellular hyphae without lysing host cells[50].*A. fumigatus* produces proteases that disrupt the actin cytoskeleton in A549 cells[51]. *A. fumigatus* also produces mycotoxins, such as gliotoxin[42], which are implicated in epithelium modifications during infection through paracellular transport, as well as transcytosis.

3.3 | Trojan Horse Mechanism

Phagocytosis is a crucial mechanism in the clearance of pathogens. Yet paradoxically, phagocytic cells can become vehicles for fungal dissemination by serving as “Trojan Horses”[52,53] (Fig. 1C), because phagocytic host cells can pass through the BBB under certain conditions. As previously described, *C. neoformans* has a thick polysaccharide capsule, which can protect yeasts against reactive oxygen species (ROS) generated by host cells[54]. The capsule enables *C. neoformans* to survive within the phagosome of human and mouse mononuclear phagocytes[55–57]. Studies have demonstrated that monocytes containing *C. neoformans* are present in the perivascular space of the brain vasculature of infected mice[53,58] and transmigrate endothelial cell layers for *C. neoformans* to gain access to the brain[59]. Depletion of monocytes or neutrophils with clodronate liposomes or Ly6G antibody, respectively, reduces cryptococcal burdens in brains of infected mice[53]. Once *C. neoformans* crosses the BBB, the fungus exits macrophages[60] and neutrophils[61] via non-lytic exocytosis without triggering host cell death-associated inflammation[60,61]. Less is known about *Candida* and *Aspergillus* dissemination through the Trojan Horse mechanisms. However, a recent *in vivo* zebrafish study supported the Trojan Horse mechanism by demonstrating dissemination of *C. albicans* yeasts by using neutrophils as vehicles to invade the CNS[62]. *A. fumigatus* can also survive within phagocytes[41], possibly due to the release of mycotoxins inhibiting macrophage oxidative burst[63]. Thus, *Candida* and *Aspergillus* may use the Trojan Horse mechanism, but further investigation is necessary to determine if these fungi use this mechanism to infiltrate the CNS.

4 | PATHOGEN DETECTION BY CNS-RESIDENT CELLS

In this section, we describe the capacity of CNS-resident cells to detect pathogens through PRRs. Although peripheral innate immune cells express PRRs, studies have shown that CNS-resident cells also express a variety of PRRs and respond to PRR signaling, including the production of chemokines and cytokines. PRRs recognize pathogen-associated molecular patterns (PAMPs), but PRRs also detect damage-associated molecular patterns (DAMPs), which are released by host cells that are injured, stressed, or died due to inflammation and tissue insults. This section focuses on PRR expression and responses by CNS-resident cells (Table 1) in fungal and other microbial infections. In some cases, we also described PRR signaling activated by endogenous molecules.

4.1 | Microglia

Microglia are CNS-resident macrophage-like cells and makeup 5–12% of brain cells in mice[64] and 0.5–16.6% of brain cells in humans[65]. Microglia are involved in various stages of CNS biology, such as CNS development, homeostasis, and neuroinflammation. Microglia also serve as CNS sentinel cells to detect infections with highly developed

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phagocytic activity as macrophage-like cells. For the origin of microglia, monocytes from the circulation were previously considered to differentiate into microglia in the CNS. However, microglia are ontogenically distinct from other mononuclear phagocytes, including peripheral macrophages, and do not arise from hematopoietic stem cells in the bone marrow[66–68]. A parabiosis approach clarified that microglia progenitors are not recruited from the circulation[68]. Instead, in mice, microglia arise from the yolk sac at embryonic day 8.5–9.5 and migrate to the brain before the closure of the BBB[66,67]. Microglia self-renew after being seeded in the brain and are maintained throughout life independently of the hematopoietic system[66,67]. Unlike other macrophages, microglia resist high-dose irradiation[69,70]. Yet, like peripheral macrophages, microglia express a broad range of PRRs, detect pathogens, participate in antigen presentation, and secrete various soluble molecules to communicate with other cells in the CNS[71]. As CNS-resident macrophage-like cells, microglia are the best-studied CNS-resident cells regarding PRRs (Table 1; Fig. 2A). However, a limited number of previous studies used *in vivo* microglia-specific mutant systems. Therefore, it is possible that some PRRs, which were previously identified in microglial tissue culture, might not be functional *in vivo,* although this issue also applies to CNS-resident cells other than microglia.

4.1.1: TLRs in microglia—Microglia constitutively express Toll-like receptors (TLRs), such as TLR1–9, TLR11–13 in mice[72,73] and TLR1–10 in humans[74,75], together with their adaptor proteins. Expression levels of various TLRs depend on the localization of microglia, age of hosts, and the condition of the CNS, *i.e*., setting of neurodegenerative diseases or infections[72,73,75,76]. Microglial TLRs have been studied as inducers of proinflammatory responses against microbial pathogens. The functions of microglial TLRs have become a focus of intensive research. Together with C-type lectin receptors (CLRs), TLRs are critical for host fungal recognition. Studies on peripheral macrophages have revealed that fungal PAMPs are recognized by multiple TLRs (i.e., TLR2/1, TLR4, TLR3, TLR2/6, TLR7, and TLR9)[77]. Among these TLRs, TLR2, 4, and 9 have been most intensively studied as detectors of fungal pathogens, including species of *Candida*, *Cryptococcus, and Aspergillus*[78–85], although further microglia-specific assessments of these TLRs will merit better understanding of CNS fungal infections.

Cell surface TLRs, including TLR4, are receptors that use Myeloid Differentiation primary response 88 (MyD88) as a key adaptor protein, which initiates activation of the transcription factors, mainly NFκB. TLR4 signaling induces the expression of proinflammatory cytokines[72]. In addition to well-known lipopolysaccharide (LPS), microglial TLR4 signaling is activated with neuraminidase (NA), a component of the envelope and membrane of some viruses and bacteria, respectively[86]. Because *C. albicans* also expresses NA[87], microglial TLR4 may also be involved in *Candida* detection. An *ex vivo* study showed that TLR4 stimulation with LPS induces proinflammatory gene expression, such as *Il1b, Il6,* and *Nos2,* in primary microglia, although the same concentration of peptidoglycan, stimulating TLR2, induced expression of these genes to a slightly lesser extent[72]. More recent studies implicated enhanced microglial pyroptosis in response to TLR4 stimulation during spinal cord injury[88]. TLR4 inhibition induced microglia to have an “M2” phenotype[89], characterized by the expression of Triggering Receptor Expressed on Myeloid Cells 2

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(TREM2) and IL-10, which may promote angiogenesis and prevent neuronal death[90]. TLR2 is also a cell surface TLR and forms a heterodimer with either TLR1 or TLR6. TLR2/1 and TLR2/6 detect triacyl lipopeptides and diacyl lipopeptides, respectively. TLR2/1 and TLR2/6 heterodimers on mouse macrophages detect GXM, a capsule component of *C. neoformans*[91]. However, *A. fumigatus* is detected only by TLR2/1 on human macrophages, despite mouse macrophages detecting *A. fumigatus* through both TLR2/1 and TLR2/6[92]. For detecting CNS endogenous molecules, microglial TLR2 detects neuron-derived α-synuclein to enhance neuroinflammation[93], where the involvement of TLR2/1 heterodimer is suggested[94]. Nevertheless, preferential formation of TLR2/1 or TLR2/6 and specific outcomes of a heterodimer have been largely unexplored in microglia.

TLR3, TLR7, TLR8, and TLR9 are localized in the endosome, recognize nucleic acids, and are extensively studied in eliciting antiviral responses through type-I interferon (IFN-I) production[95]. TLR3 detects double-stranded RNA (dsRNA) and depends on TRIF for downstream signaling as endosomal TLR4 does, while other endosomal TLRs require MyD88 for eliciting their signaling. *Tlr3* mRNA is constitutively expressed in microglia[96]. Primary microglia in culture respond to TLR3 stimulation with poly(I:C), by inducing gene expression, including *Tlr3* and *Ifnb,* and secreting Tumor Necrosis Factor alpha (TNFα), IL-6, and IL-12p40[97]. Microglia in *Tlr3*$^{-/-}$ mice did not undergo microgliosis, an intensive reaction of microglia to pathogenic insults, after a poly(I:C) intracerebroventricular (*i.c.v.*) injection, while in wild-type mice, the treatment induced microgliosis[97]. This study strongly suggested that TLR3 on microglia is biologically functional *in vivo*[97]. Unexpectedly, *Tlr3*$^{-/-}$ mice are more resistant than wild-type mice to encephalitic viral infections by West Nile virus (WNV) and a specific setting of Theiler's murine encephalomyelitis virus infections[96,98]. The detrimental role of TLR3 in WNV infection, at least, could be explained by TLR3-dependent inflammatory response, which allows CNS infiltration of WNV and neuronal injury[96]. Although the role of microglial TLR3 is not known in fungal infections, dsRNA from *A. fumigatus* conidia activates lung epithelial cells through TLR3[99]. Thus, TLR3 on microglia may also detect *A. fumigatus* conidia. However, it is unknown if microglial TLR3 is a beneficial or detrimental PRR in fungal infections.

Functions of other TLRs in microglia are still largely unexplored in fungal infection settings. Here, we briefly describe previous studies on roles of the microglial TLRs. The following studies were not performed in the context of fungal infections, but the findings might be extrapolated to possible outcomes when these TLRs are stimulated by fungi. TLR7 detects single-stranded RNA (ssRNA), and its expression has been observed in human[76] and mouse microglia[100] in numerous neurodegenerative diseases. *In vivo* administration of R848, a TLR7 agonist, induces acute sickness associated with CNS proinflammatory gene expression and microglia morphology changes[101]; thus, TLR7 is functional in microglia *in vivo*. TLR8 also recognizes ssRNA and is structurally related to TLR7. TLR8 is expressed in microglia[72], but its function has yet to be addressed. TLR9 detects microbial and endogenous dsDNA. Previous studies suggested that TLR9 stimulation has substantial impacts on the CNS[102–105], but it is still unknown if and how microglial TLR9 is involved in fungal infections. Additionally, although it is clear that TLR9 is expressed and functional in microglia, experimental systems to clarify the involvement of microglia-specific TLR9, such as using TLR9 conditional knockout mice, will clarify the implication of TLR9 stimulation

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in microglia *in vivo*. Other microglial TLRs reported include TLR5, which detects bacterial flagellin. TLR5 expression has been described in human and mouse microglia[72,76], and its functional relevance has been explored in CNS disorders such as neuropathic pain, stroke, and Alzheimer's disease (AD)[106–108]. *Ex vivo* microglial TLR5 stimulation with flagellin results in the release of proinflammatory cytokines, promoting chemotactic microglial migration, and enhancement of phagocytosis[109]. TLR10 is a human receptor, and its expression and function in microglia remain a subject for further research. TLR11–13 are murine TLRs, and their expression is elevated during murine neurocysticercosis, a parasitic infection that causes neurologic syndromes such as epileptic seizures[73,110,111], but their functions in microglia remain unknown.

4.1.2: CLRs in microglia—Microglia also highly express a variety of CLRs, expressed primarily by myeloid cells (Table 1; Fig. 2A). In peripheral immune cells, dectin-1, dectin-2, MCL/dectin-3, Mincle, mannose receptor (MR), and Dendritic Cell-Specific Intercellular adhesion molecule-3-Grabbing Non-integrin (DC-SIGN) have been studied as detectors of fungal pathogens[112]. Among them, microglia express dectin-1, Mincle, MR, and DC-SIGN[113–116]. Dectin-2 does not appear to be expressed at detectable levels in human or murine microglia[117]. Dectin-1, encoded by the *Clec7a* gene, was the first CLR identified in macrophages to detect fungi[118,119] through detection of β-glucans, cell wall components of fungi, some bacteria, and plants[120]. Dectin-1 on macrophages collaborates with TLR2 and MR for heightened fungal sensing[121]. Although dectin-1 is not required for detecting *C. neoformans*[122], it is crucial to recognize *Candida* and *Aspergillus*[118,123]. *Clec7a*$^{-/-}$ mice infected with *C. albicans* showed increased fungal loads in the brain[124]. Thus, dectin-1 limits brain fungal loads in *C. albicans* infection but not in infections by *C. glabrata, C. tropicalis,* and *C. parapsilosis*[124], suggesting that the role of dectin-1 in *Candida* is specific to species. Interestingly, a study showed that β-glucan stimulation is not sufficient for substantial production of cytokines or chemokines by microglia, although ROS generation and phagocytosis are actively induced[125]. In particular, the involvement of vav guanine nucleotide exchange factor 1 (Vav1) and phosphatidylinositol 3-kinase (PI3K) was identified in ROS generation by microglia[126].

Dectin-1 is a critical receptor in eliciting host responses against fungal infections. Therefore, this section is intended to discuss microglial dectin-1 in neuropathological settings other than fungal infections. Clarifying if dectin-1 signaling in microglia shares common outcomes and mechanisms between CNS mycosis and CNS sterile inflammation will merit further studies. Recent studies have revealed the involvement of dectin-1 on microglia in various sterile and non-homeostatic settings. Microglia, in mice at least, have no or very low expression of dectin-1 during homeostasis, but its expression is significantly upregulated during neurodevelopment and under CNS pathological conditions[113,127–130] (more details described in our previous review article[131]), and its expression is tightly upregulated in the spinal cord of mice with experimental autoimmune encephalomyelitis (EAE)[113] and in the brain of aged APP-PS1 mice with β-amyloid accumulation[127]. Indeed, recent studies reported subsets of microglia, including microglial neurodegenerative phenotype (MGnD) and disease-associated microglia (DAM), highly express *Clec7a* in the brains of mice and humans in neuroinflammatory and neurodegenerative diseases[127–129]. Dectin-1-expressing

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microglia are associated with β-amyloid plaques and diffuse neuritic dystrophy in the cortex of AD model mice[127]. In addition, *Clec7a* upregulation was also identified in early postnatal proliferative region-associated microglia (PAM) during sterile CNS development[130]. PAM exhibit amoeboid morphology, are metabolically active, and phagocytose newly formed oligodendrocytes[130]. Thus, dectin-1 is highly expressed in microglia during pathological and neurodevelopmental settings independent of microbial infections[131]. Dectin-1 could function as an immunoregulatory receptor by detecting endogenous molecules in non-infectious settings[113,132], and the immunoregulatory role of dectin-1 might become simultaneously active with the canonical proinflammatory role of dectin-1 during CNS fungal infections.

DC-SIGN in peripheral antigen-presenting cells recognizes mannose-containing glycoproteins on the surface of pathogens, such as *C. albicans, Mycobacterium tuberculosis*, and *Helicobacter pylori*[133]. Human microglia also express DC-SIGN[116]. Other studies demonstrated that DC-SIGN serves as a co-receptor for several viruses[134,135]. Unstimulated microglia show little to no expression of DC-SIGN, but DC-SIGN is detected in primary human microglia stimulated *ex vivo*[116,136]. DC-SIGN on dendritic cells (DCs) and microglia recognizes myelin, which is rich in carbohydrates and mediates internalization and endolysosomal routing of large myelin particles[116]. Costimulation of DC-SIGN and TLR4 enhances DC IL-10 production upon detecting myelin oligodendrocyte glycoprotein (MOG)[116]. The IL-10-producing immunoregulatory effect by DCs was abrogated when myelin had less fucosylated glycans present[116]. Thus, DC-SIGN may also play an immunoregulatory role in CNS fungal infection.

4.1.3: NLR in microglia—The nucleotide-binding oligomerization domain (NOD)-like receptors (NLRs) are intracellular sensors. The NOD subfamily of NLRs include NOD1 and NOD2, which recognize peptidoglycan motifs found mainly in bacteria. Expression of NOD1 and NOD2 was identified in microglia[137,138]. In pulmonary aspergillosis, NOD1 is detrimental to hosts by limiting host defense against *Aspergillus*[139], but NOD2 is beneficial for host resistance[140]. NOD2 is also critical for the inflammatory response against neurotropic bacteria[141,142].

The Nucleotide-binding oligomerization domain, Leucine-rich Repeat and Pyrin domain containing (NLRP) subfamily consists of intracellular sensors that mediate inflammasome activation by sensing a wide range of PAMPs and DAMPs. In particular, NLR Family Pyrin Domain Containing 3 (NLRP3) and NLR Family CARD Domain Containing 4 (NLRC4) inflammasomes detect *Candida, Cryptococcus, Aspergillus,* and *Paracoccidioides* (reviewed in[143]). Microglia express components necessary for the formation of inflammasomes, including NLRP1, NLRP3, pro-caspase-1, and ASC[144]. Expression of inflammasome components alone does not necessarily mean inflammasomes are activated or functional, because polymerization of inflammasome components is essential for inflammasome activation. For example, our lab recently reported that inflammasomes are not activated in microglia in the spinal cord during EAE[145], although expression of NLRP3 inflammasome components is detected in microglia. Due to the technical limitation in detecting active inflammasomes in the CNS, most previous studies stopped short of demonstrating *in vivo* or *in situ* inflammasome activation in microglia and other CNS cells. Instead, many studies described the expression of inflammasome components or inflammasome activation

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ex vivo. For example, the NLRP3 inflammasome is activated *ex vivo* in microglia in response to lysophosphatidylcholine (LPC), a molecule associated with neurodegeneration and demyelination[146]. In a model of ischemia, NLRC5 is upregulated in microglia and directly binds to NLRP3 and NLRC4 in a nucleotide-binding domain-dependent manner to cooperatively drive microglial pyroptosis[147].

4.1.4: RLRs, and other PRRs in microglia—RIG-I-like receptors (RLRs) are cytosolic RNA sensors, which trigger the production of IFN-I. The RLR family is composed of three members: retinoic acid-inducible gene I (RIG-I), melanoma differentiation-associated protein 5 (MDA5), and laboratory of genetics and physiology 2 (LGP2). Isolated mouse microglia constitutively express RIG-I and MDA5 transcripts and proteins[148]. Although RLRs have been extensively studied in the context of antiviral immunity, the involvement of RLRs in antifungal immunity has only recently been reported. One report using mouse macrophages and human PBMCs has shown that the yeast-to-hyphae transition of *C. albicans* induces expression of *IFIH1*, encoding for MDA5[149]. The study also showed that MDA5-deficient splenocytes failed to recognize *C. albicans* hyphae, resulting in reduced IFNβ expression[149], suggesting recognition of *C. albicans* hyphae by MDA5. Furthermore, patients suffering from chronic mucocutaneous candidiasis have lower levels of *IFIH1*, suggesting the involvement of MDA5 in systemic candidiasis[149]. Based on the constitutive expression of MDA5 in microglia[148], the involvement of microglial MDA5 in CNS candidiasis is expected.

AIM2-like receptors (ALRs) usually contain an N-terminal pyrin (PYD) domain, and one or two HIN domains. Absent In Melanoma 2 (AIM2) is a cytoplasmic DNA sensor, which forms an inflammasome upon detecting exogenous and endogenous dsDNA. Although the study focused on DCs in mice, *A. fumigatus* induces the formation of the AIM2 inflammasome complex, which also includes NLRP3 and caspase-8, in addition to canonical inflammasome components, ASC and caspase-1[150]. With this mechanism, the AIM2 inflammasome protects hosts from pulmonary aspergillosis[150]. Microglia express AIM2, and stimulation with poly(dA:dT) induces IL-1β and IL-18 release from microglia[151], suggesting that the AIM2 inflammasome can be activated in microglia *ex vivo*. A more recent study showed that AIM2 in microglia limits EAE in an inflammasome-independent mechanism by negatively regulating cyclic GMP-AMP synthase (cGAS) and the DNA-dependent protein kinase (DNA-PK) pathways[152]. The study also demonstrated in an EAE setting that AIM2-deficient microglia induce the expression of genes related to immune responses against virus infections[152]. Thus, the study suggested that AIM2 in microglia negatively regulates inflammatory immune responses without forming an inflammasome. It is unknown if the inflammasome-independent AIM2 in microglia is functional in CNS fungal infections.

The cGAS- Stimulator of Interferon Genes (STING) pathway senses cytosolic dsDNA and induces IFN-I production by IRF3 activation. Although the study focused on human corneal epithelial cells (HCECs) and mouse corneas, cGAS-STING contributes to the progression of *A. fumigatus* keratitis[153]. Yet, it is not known which source of DNA, host or *A. fumigatus,* stimulates the cGAS-STING pathway. Another study identified the cGAS-STING pathway activation in Iba1-positive cells in the brain of mice in the middle cerebral artery occlusion

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(MCAO) model[154], suggesting the possible presence of the cGAS-STING pathway in microglia. Using a microglia BV2 cell line, the study also demonstrated the activation of cGAS-STING to unspecified DAMPs[154]. Taken together, it is plausible that cGAS-STING activation in microglia occurs during CNS infection, but this possibility remains to be addressed.

4.2 | Astrocytes

Arising from primary neural stem cells, astrocytes are the most abundant cell population in the CNS and contribute to CNS functions in health and disease. During CNS homeostasis, astrocytes are critical cells for the formation and maintenance of the BBB; controlling extracellular fluid, ions, and neurotransmitters; providing energy substrates to neurons; modulating blood flow; and regulating drainage of interstitial fluid[155–158]. Astrocytes also have roles in synapse development and plasticity[159], and interact with various cells in the CNS. Despite significantly fewer reports on pathogen sensing by astrocytes than microglia, previous studies demonstrated the involvement of astrocytes in shaping the immunological milieu upon microbial infections and neurodegeneration[113,160,161]. In these pathological conditions, astrocytes undergo morphological and functional changes in a process known as reactive astrogliosis, in which the most common hallmarks include upregulation of glial fibrillary acidic protein (GFAP), the main component of astrocyte intermediate filaments and hypertrophy of astroglial processes[162]. During this process, astrocytes can form a glial scar around lesions during trauma or infection[163]. Additionally, astrocytes produce cytokines, chemokines, and growth factors, although astrocytes express less variation of PRRs than microglia (Table 1; Fig. 2B). Technical challenges in astrocyte handling, such as obtaining a highly pure astrocyte population and their susceptibility in *ex vivo* environments, hinder functional analyses of astrocytes. Their functions during fungal infections remain largely unknown. At least, the formation of an astrocytic border around lesions has been demonstrated in several mouse models of fungal infections[28,164], although direct interactions between astrocytes' PRRs and fungi have not been elucidated.

4.2.1: TLRs in astrocytes—Roles of astrocytic TLRs in fungal infections have yet to be explored, and limited information is available. In this subsection, we discuss previous findings, which provide knowledge to be considered when the roles of astrocytic TLRs in fungal infections are studied. The majority of TLR studies with astrocytes were performed *ex vivo* and focused on TLR expression or release of cytokines and chemokines by astrocytes in response to canonical TLR ligands. TLR expression under the homeostatic condition is reported in humans (TLR1–5, TLR9) and mice (TLR1–9 and TLR11–13) astrocytes[75,165,166] (Table 1). As discussed below, astrocytes can substantially upregulate TLR expression levels; thus, TLRs in astrocytes may work secondary to PAMP detection by microglia, which are equipped with relatively high levels of TLRs without cell stimulation[167].

TLR3 expression in human astrocytes is induced with various stimuli, including agonists of TLR3 and TLR4 and cytokines, such as TNFα, IL-6, IFNγ, and IL-12[168]. In contrast, IL-10, an anti-inflammatory cytokine, does not induce TLR3 expression[168]. After TLR3 stimulation, the expression of neuroprotective factors and anti-inflammatory cytokines, such

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as IL-10, are upregulated, and proinflammatory cytokine expression, such as IL-12p40 and IL-23, is downregulated in a human astrocyte tissue culture setting[168]. This results in inhibition of astrocyte growth, promotion of endothelial cell growth, and enhanced neuronal survival[168]. In an *in vivo* brain ischemia mouse model by MCAO, TLR3 agonist poly(I:C) attenuated reactive astrogliosis, reduced brain infarction volume, and improved neurological function[169]. These studies suggested that TLR3 in astrocytes could serve as an immunoregulatory receptor. However, another study indicated that TLR3 stimulation with poly(I:C) induced protein expression of TLR2, IL-6, C-C Motif Chemokine Ligand 5 (CCL5), Intercellular Adhesion Molecule 1 (ICAM-1), and Vascular Cell Adhesion Molecule 1 (VCAM-1) by primary mouse astrocytes in tissue culture[170], suggesting TLR3 stimulation enhanced inflammation. The same study also demonstrated that TLR3-stimulated astrocytes slightly triggered antigen-specific $CD4^+$ T cell activation *ex vivo* based on the evaluation of T cell proliferation and IFNγ production[170]. A more recent study indicated that human astrocytes treated with IFNγ (although TLR3 agonist was not tested), enhanced the expression of MHC class I and II, as well as co-stimulatory molecules (CD80/86/40)[171]. These studies suggest the involvement of TLR3 in modulating the CNS environment. However, further study is required to determine the involvement of astrocytic TLR3 in antigen-presentation *in vivo* as new antifungal host responses.

An early study using primary mouse astrocytes showed expression of *Tlr2, 4, 5,* and *9* and demonstrated that their gene expression levels could also be induced[172]. Various stimuli can induce TLR2 expression on astrocytes. For example, PGN, flagellin, and CpG-DNA highly induce *Tlr2* expression in mouse astrocytes *ex vivo*[172]. Bacterial infection with *Brucella melitensis* also enhanced TLR2 expression in the white matter of rhesus macaques[173]. TLR2 on astrocytes recognizes *Streptococcus suis*, which causes meningitis, and induces expression of TLR2, TLR6 (but not TLR1), and a downstream proinflammatory response[174,175]. Another study showed that TLR2 expression on astrocytes depends on TNFα and NFκB pathways[176]. Multiple studies using primary astrocyte tissue culture demonstrated functional outcomes of TLR2 signaling on astrocytes. For example, stimulation of TLR2 with lipoteichoic acid induces the expression of matrix metalloproteinase 9 (MMP-9) in rat astrocytes[177]. *Ex vivo* treatment of mouse primary astrocytes with PGN triggers expression of TNFα, C-X-C Motif Chemokine Ligand 2 (CXCL2), and CCL4, and the involvement of TLR2 in the response was confirmed with $Tlr2^{-/-}$ astrocytes[176]. However, priming may be required for TLR2-mediated functions in astrocytes because TLR2 signaling needed pre-treatment of astrocytes with proinflammatory cytokines, such as TNFα, IFNγ, and IL-1β, for overt TLR2-mediated responses[167]. The study used primary mouse astrocytes in tissue culture[167]. Sensitization of TLR2 can also be achieved with conditioned media from microglia culture[167], suggesting that astrocytes require crosstalk with microglia or CNS-infiltrated inflammatory cells to efficiently exert outcomes of TLR2 stimulation. For astrocytic TLR2 signaling activation, astrocyte priming may be necessary to induce TLR2 expression to a certain level.

TLR4 on astrocytes appears to be ready to detect ligands because of its constitutive expression. *Tlr4* mRNA expression levels without stimulation are already higher than those of *Tlr1, Tlr2,* and *Tlr3* in human astrocytes[168]. A study showed that LPS stimulation of primary murine astrocytes induced gene expression of *Tnf, Il1b, Il6, Cxcl1,*

Cxcl2, Ccl2, Ccl7, and *Ccl12* as early as 2 hours after stimulation[178], suggesting that TLR4 was present on astrocytes and able to detect LPS[178]. Other studies also showed functional TLR4 on astrocytes without pre-treatment. For example, LPS stimulation of murine astrocytes activates NFκB, MAPKs, and JAK/STAT signaling pathways[179], and enhances the expression of inducible Nitric Oxide Synthase (iNOS), IL-6, CCL2, ICAM1, and VCAM1[170]. Yet, earlier studies showed conflicting results. One study concluded that astrocytes (and oligodendrocytes) do not express *Tlr4* mRNA, despite the positive *Tlr4* detection in microglia, using primary cells from newborn rat forebrains without stimulation[180]. The study[180] was published in 2002; therefore, the detection of cDNA was performed on an agarose gel, rather than quantitative PCR. Thus, it is possible that detection was not sensitive enough to detect *Tlr4* transcripts. In contrast, another study published in 2003 also used agarose gels and clearly showed the *Tlr4* cDNA band, although astrocyte stimulation was necessary for visualizing solid *Tlr4* cDNA bands[172]. A different study showed a very low TLR4 protein expression level in resting mouse astrocytes when detected by flow cytometry[170]. Here, it is possible that TLR4 detection by flow cytometry might have been technically challenging if the TLR4 antibody was not sensitive, and the absence of a positive TLR4 staining control prevented evaluation of the TLR4 antibody sensitivity[170]. Mouse astrocytes show only mild induction of *Tlr4* gene activation with various stimulations, such as LPS, poly(I:C), PGN, flagellin, and CpG-DNA[170,172]. The mild induction of *Tlr4* is a substantial contrast to that of *Tlr2* and *Tlr3*[170]. *Tlr4* gene expression in human primary astrocytes is even more resistant to stimulation with inflammatory cytokines, such as IL-1β, TNFα, and IFNγ[168]. These basal TLR4 expression levels may be low enough to be missed, and *Tlr4* expression is relatively resistant for upregulation. However, it appears that astrocytes can express functional TLR4.

Expression of TLR7 and TLR9 in astrocytes is inducible by ZIKA virus infection[181]. TLR7 agonist, imiquimod, reactivates astrocyte and induces production of proinflammatory cytokines and chemokines, including IFNβ, TNF, CCL2, and CXCL10[182]. *Ex vivo* TLR9 stimulation of mouse astrocytes with its ligand, CpG-DNA, upregulates the expression of cathelin-related antimicrobial peptide[183], suggesting that TLR9 signaling is functional in astrocytes. Although ligands and functions for TLR11–13 in astrocytes remain to be addressed, toxoplasmosis in mice upregulates the expression of TLR11 in astrocytes[184].

4.2.2: CLRs in astrocytes—CLR expression by astrocytes appears to be limited. For example, the expression of “Aplec” genes (the “Antigen-Presenting lectin-like receptor gene complex” and including a cluster of C-type lectin (CLEC) receptor gene loci), including *Clec4e* (encoding Mincle), was not detected in rat astrocytes, while microglia express them when stimulated[185]. To the best of our knowledge, no reports showed astrocytic dectin-1 and other major CLRs, involved in fungal detection. Yet, MR is highly expressed in astrocytes in one week-old neonatal mice and decreases its expression level to maintain low levels throughout adulthood[186]. Mannose-binding lectin (MBL) is detected in astrocytes of post-mortem brain tissue of patients with HIV encephalitis[187]. These studies suggest that astrocytes may not be equipped to directly detect fungal infections through CLRs, although our understanding of CLRs in astrocytes is still not sufficient for this conclusion.

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4.2.3: NLRs and ALRs in astrocytes—Astrocytes are known to express some NLRs and AIM2 inflammasome components. For NLRs, NOD1 and NOD2 are expressed in astrocytes[188]. NOD2 in retinal astrocytes has been shown to synergize with TLR2 for the recruitment of pathogenic T cells during intraocular inflammation[141,142,189]. NLRP3 and NLRC4 inflammasomes are activated in LPC-stimulated mouse primary astrocytes *ex vivo*, and resulting IL-1β secretion was reported[146], suggesting that astrocytes could behave as inflammatory cells through these inflammasomes. Similarly, *ex vivo* treatment of mouse primary astrocytes with nigericin, an NLRP3 inflammasome stimulator, also allows IL-1β secretion, although poly(dA:dT)/liposome, an AIM2 inflammasomes stimulator, fails to secrete detectable levels of IL-1β[145]. Human primary astrocytes express NLRP2, which can be activated by adenosine triphosphate (ATP) as a DAMP, and process pro-caspase-1 and pro-IL-1β[190]. Based on these studies, astrocytes appear to have the capacity to activate various inflammasomes, including NLRs.

Our lab recently reported that astrocytes can also have activated AIM2 inflammasome in the spinal cord during a late stage of EAE[145]. Unexpectedly, astrocytes are the main CNS cell type exhibiting AIM2 inflammasome "activation", and neither microglia nor CNS-infiltrated myeloid cells are sources of any active inflammasomes during EAE[145]. Also, the NLRP3 inflammasome is not activated in astrocytes, contrasting to the NLRP3 inflammasome activation and its pathogenic role in EAE in peripheral myeloid cells[145,191–193]. Interestingly, mouse primary astrocytes with active AIM2 inflammasome release little IL-1β due to globally limited expression of inflammasome components[145]. The function of the AIM2 inflammasome in astrocytes is not inflammatory based on limited IL-1β production by astrocytes and more severe EAE in *Aim2*$^{-/-}$ mice[145,152,194]. Yet, the biological role of active AIM2 inflammasome in astrocytes during EAE is still unknown. Taken together, the roles of activated inflammasomes in astrocytes *in vivo* are still largely elusive, particularly due to its generally low expression levels of inflammasome components[145]. Nevertheless, NLR inflammasomes in astrocytes appear to induce inflammation, while the AIM2 inflammasome in astrocytes may regulate inflammation, or at least its activation is not inflammatory.

4.2.4: *RLRs* in astrocytes—Reports have shown that RIG-I and MDA5 are expressed in rat, human, and murine astrocytes[195–197]. While many studies focus on understanding their roles in CNS injury, few reports address their functionality during infections. Cultured astrocytes in a stretch injury model showed elevated RIG-I and MDA5 expression, leading to the production of IFN-I and an increase in GFAP and vimentin, hallmarks of reactive astrogliosis[195]. RIG-I expression in astrocytes is also inducible by hypoxia and infection by vesicular stomatitis virus (VSV)[196,197]. In the VSV study, primary human astrocytes produce inflammatory cytokines and neurotoxic mediators[197]. In mouse astrocytes, cytosolic poly(I:C) complexed with Lipofectamine activate MDA5, but not RIG-I, and induces the release of IFNβ and IL-6[198].

Astrocytes have functional STING, although the setting in a report did not directly sense cytoplasmic DNA from the astrocyte nucleus[199]. Rather, STING was activated by cyclic guanosine monophosphate–adenosine monophosphate (cGAMP), transferred from brain metastatic cancer cells through gap junction channels with connexin-43 (Cx43) through cell-

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cell contact[199]. In addition, the astroglial STING pathway activation elicited the production of IFNα and TNF[199]. This intriguing study suggests a possible role of astrocytes as secondary sensors of DNA damage in other CNS-resident cells, such as injured neurons, as well as injured astrocytes, known to be physically connected with healthy astrocytes through Cx43[200]. The cGAMP-STING signaling in bystander cells is also reported with murine embryonic fibroblasts (MEFs) during virus infection[201]. In this case, volume-regulated anion channels (VRACs) play a role in cell-to-cell transmission of cGAMP to enhance antiviral IFN-I response[201].

Although astrocytes are not well equipped with various PRRs, compared to microglia with their extensive PRR repertoire, further studies are merited to identify the roles of astrocytes as a primary or secondary cell type to sense infections or even as amplifiers or regulators of infection-triggered cell responses by CNS immune sentinels.

4.3 | Neurons

Neurons are fundamental cells in the CNS and peripheral nervous system (PNS) responsible for the relay of information throughout the body. Some neuronal PRRs are used to detect infections, but a majority of previous studies focused on the involvement of PRRs in neurons in the light of neuronal death and growth (Table 1; Fig. 2C).

4.3.1: TLRs in neurons—Neuronal TLRs have been studied mainly in the context of neurodegenerative diseases and neurodevelopment. Several studies reported the expression of TLR1–9 in neurons in the CNS and the PNS[166,202–207]. Interestingly, some neuronal TLRs may function through glycogen synthase kinase 3β (GSK3β), jun-N-terminal kinase (JNK), and PI3K/protein kinase B (AKT) pathways, rather than the canonical NFκB pathway[204,208,209]. Expression of TLR2 and TLR4 in neurons is increased in response to IFNγ stimulation and energy deprivation, as well as in response to ischemic brain injury[204]. The study also reported that TLR2 and TLR4 in neurons enhance proapoptotic signaling, rendering neurons vulnerable to ischemic death[204]. TLR4 in neurons may have a distinct role or different expression pattern from TLR2 in neurons in AD. For example, only TLR4 expression was increased in neurons when exposed to amyloid β (Aβ) peptide, despite both TLR4 and TLR2 being upregulated in cultured neurons upon exposure to hydoxynonenal (HNE), an AD-related byproduct[210]. TLR4 is also responsible for JNK phosphorylation and caspase-3 cleavage, resulting in neuronal death, upon HNE treatment[210]. This suggests that TLR4 signaling in neurons may be detrimental during AD; but the functional consequence of TLR2 in neurons remains undefined in this setting. Nevertheless, neuronal TLR2 is required for opioid-induced neuronal death via the activation of the GSK3β signaling pathways[209], as well as glucose deprivation-induced cell death[204]. Thus, TLR2 and TLR4 signaling are generally neurotoxic. If fungi stimulate neuronal TLR2 and TLR4, it may be detrimental to neurons rather than eliciting antifungal responses.

TLR3 and TLR5 in primary sensory neurons are involved in controlling itch and pain[211,212]. For example, a TLR3 agonist induces action potentials in dorsal root ganglion (DRG) neurons and elicits scratching in wild-type mice but not in TLR3-deficient mice[206]. TLR5 in DRG neurons was suggested to be a target to treat neuropathic pain through the blockade of

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Aβ-fibers, which are implicated in mechanical allodynia[106]. These studies show that TLR3 and TLR5 regulate neuronal excitability and synaptic transmission.

TLR7 is expressed in neurons, and *in vivo* neuronal cell death is induced by ssRNA40, an oligoribonucleotide from HIV[205]. TLR8 in neurons is a negative regulator of neurite growth and an inducer of neuronal apoptosis[213]. The study also reported that stimulating TLR8 in neurons with R848 (also a TLR7 agonist) downregulates phosphorylated IκB without impacting NFκB p65 activation[213]. TLR9 in spinal cord neurons is considered involved in the excitotoxic death of neurons, at least partly due to stress in the endoplasmic reticulum[214]. Another study showed that some neurons in mice express TLR11 after encephalitic *Toxoplasma gondii* infection[184], but the role of TLR11 is unknown.

4.3.2: CLRs in neurons—Spinal cord neurons express low levels of Mincle and induce Mincle expression in response to peripheral nerve injury (PNI). Mincle signaling in neurons appears to induce TNFα production and allodynia induced by PNI[215,216]. Neurons also express MR under certain conditions[186], but its functional relevance in neurons remains undetermined. MBL is expressed in human neurons with increased expression during HIV-encephalitis, but the function of MBL is still not clear[187]. The roles of CLRs during fungal infections remain unaddressed.

4.3.3: NLRs and ALRs in neurons—NOD1 is highly expressed in neurons of developing mouse brain, and it may function to recognize microbiota-derived PGN that cross the BBB during brain development[188]. Rat cortical neurons also highly express NOD1 by an *ex vivo* oxygen-glucose deprivation and reperfusion treatment[217]. Among this PRR category, neurons have been relatively well studied in the light of inflammasomes. Neurons from naïve mice express detectable levels of NLRP1, caspase-1, and ASC proteins[218]. Human cortical brain neurons also showed the expression of NLRP1, caspase-1, ASC, NLRC4 (IPAF), and AIM2, although NLRP3 expression was not identified[219]. The study demonstrated that *ex vivo* treatment of human neurons with muramyl dipeptide (MDP), flagellin, and poly(dA:dT) activated caspase-1 in neurons, suggesting functional NLRP1, NLRC4, and AIM2 inflammasomes[219]. Furthermore, IL-1β release from neurons by NLRP1 inflammasome activation induced neuroinflammation, leading to caspase-6-mediated axonal degeneration and $A\beta_{42}$ overproduction[219]. An earlier *ex vivo* study showed NLRP1 overexpression in rat primary cerebellar granule neurons led to apoptosis, accompanied by caspase-3 activation, resulting in neuron injury[220]. In contrast, a non-inflammasome forming NLR, NLR Family Member X1 (NLRX1), protects neuron-like N2A cells from necrosis[221].

AIM2 expression has also been reported in human neuron cultures[219]. The AIM2 inflammasome is activated in rat embryonic cortical neurons, based on the detection of pro-caspase-1 processing, along with the formation of "ASC specks," which are microscopically detectable signals reflecting the inflammasome polymer complex formation[222]. A more recent study indicated the detection of *Aim2* transcripts in neurons, along with astrocytes and microglia, during brain development in mice[223]. AIM2 total knockout mice demonstrated increased DNA damage accumulation, which was also identified in neuron-specific caspase-1 knockouts[223]; thus, ASC specks detected in the brain development appear to be activated by AIM2 inflammasome in neurons. Indeed, we identified activated AIM2

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inflammasome was also detected in motor neurons expressing Choline Acetyltransferase (ChAT) during a late stage of EAE, although the levels of activated AIM2 inflammasome in neurons are significantly lower than those in astrocytes[145].

4.3.4: RLRs and other PRRs in neurons—Neurons express RLRs in various mammalian models but mainly in the context of viral infections[208,224–226]. Expression of RIG-I and MDA5 in cultured human and rat primary neurons increases in response to IFN-I; and brain infection by simian immunodeficiency virus also enhances the expression of RIG-I and MDA5 in neurons of macaques[208,224]. Furthermore, RIG-I signaling in neurons induces inflammatory molecules, including IL-6, IL-12p70, CCL2, CXCL10, and TNFα, upon Japanese encephalitis virus (JEV) infection[225]. A later study showed that RIG-I and STING interact to elicit immunological responses involving IFN-I[226]. Together, these reports suggest that neurons are sensitive to detecting viruses through RLRs and elicit antiviral immune responses. However, the roles of neuronal RLRs in the context of fungal infections are still unknown.

4.4 | Oligodendrocytes and Oligodendrocyte Precursor Cells (OPCs)

Oligodendrocytes are responsible for maintaining and generating the myelin sheath that surrounds axons. They also support neurons by producing neurotrophic factors, which maintain axonal integrity and propagate the action potential along axons. Due to their importance in protecting neurons, oligodendrocytes have become a target of research in neurodegenerative disorders.

To the best of our knowledge, reports on PRR expression in oligodendrocytes and OPCs are limited to a few TLRs (Table 1; Fig. 2D). Among TLRs, oligodendrocytes express TLR2, and the expression is upregulated in MS lesions[227]. TLR2 stimulation by its agonists inhibits OPC maturation, while agonists of other TLRs do not[227]. Expression of TLR2, and TLR3 to a lesser extent, was also identified in primary rat oligodendrocytes culture[228]. TLR3 agonist, poly(I:C), induces apoptosis in oligodendrocytes, while zymosan promotes survival, differentiation, and myelin-like membrane formation in oligodendrocytes[228]. Although the study describes zymosan as “a TLR2 agonist”[228], zymosan is a yeast cell wall derivative and stimulates dectin-1 together with TLR2 as well. Thus, dectin-1 signaling may also be involved in the outcomes in zymosan-treated oligodendrocytes, although dectin-1 was not discussed[228]. TLR4 was also identified on OPCs and triggers apoptosis of OPCs with Hsp60, a DAMP released by microglia[229], through the TLR4-MyD88-NFκB signaling pathway[229]. Another study showed that LPS-mediated TLR4 stimulation is detrimental and toxic for oligodendrocytes in culture[180].

Information on CLRs, NLRs, RLRs, and ALRs in oligodendrocytes and OPCs is limited, except for the study on zymosan-treated oligodendrocytes as mentioned above, although the possible involvement of dectin-1 was not addressed[228]. Other studies showed that oligodendrocytes do not express MR[186], but MBL is a CLR expressed by oligodendrocytes of the frontal cortex of HIV-1 infected brains, although the role MBL in oligodendrocytes remains unknown[187].

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5 | RECRUITMENT OF BLOOD-BORNE IMMUNE CELLS TO THE CNS DURING FUNGAL INFECTIONS

Recruitment of immune cells from circulation is essential for fungal clearance in the CNS. Having CNS-infiltrated cells in the CNS adds a broader variety of PRRs and effector functions, including the secretion of cytokines and chemokines, as well as phagocytosis and killing of fungi. However, heightened immunity by CNS-infiltrated cells could cause irreparable damage to the CNS, due to relatively poor regenerative capacity of CNS-resident cells. In addition, as discussed in Section 3, recruited immune cells can contribute to fungal dissemination as vehicles of the Trojan Horse mechanism. Here, we discuss peripheral immune cells recruited to the CNS during fungal infections and their contribution to fungal clearance and CNS pathology.

5.1 | Neutrophils

Neutrophils serve as the first line of defense against fungal infections and can eliminate pathogens through various mechanisms, including phagocytosis, secretion of antimicrobial factors, generation of oxidative bursts, and release of neutrophil extracellular traps (NETs)[230]. In the absence of neutrophils, a condition known as neutropenia, individuals face a higher risk of developing invasive candidiasis[231] and aspergillosis[232]. In contrast, neutrophil-mediated control of fungal infections is known to cause immunopathology, at least in the peripheral organs[233,234]

During disseminated candidiasis, neutrophils are recruited and accumulate in the mouse brain early during infection[235]. The number of neutrophils recruited in the brain peaks at 4 days after *C. albicans* infection, and the kinetics of neutrophil counts in the brain contrasts with a steady increase in the kidney up to day 7[235]. Thus, even in the same mouse, the brain appears to limit neutrophil infiltration to a certain point. Recruitment of neutrophils to the CNS during invasive candidiasis relies on Caspase Recruitment Domain Family Member 9 (CARD9) in microglia[236], an adaptor protein downstream of Syk-mediated CLR signaling. CARD9 in neutrophils is also required to produce chemokines that further recruit monocytes and additional neutrophils[236]. Neutrophils also play a role to limit the transition from the hyphal form to the yeast form[237].

Cryptococcus infection models using mice[238–240] and zebrafish[241] showed that neutrophils are the first cells to interact with *Cryptococcus* in the brain vasculature. Complement C5a receptor signaling is critical for neutrophil recruitment to the brain vasculature, in which neutrophils are the primary cell type to clear *C. neoformans*[240]. The clearance of *C. neoformans* in the brain microvasculature by neutrophils is a crucial step for host protection; thus, increasing neutrophil recruitment to clear arrested cryptococci in the brain microvasculature was proposed as a therapeutic approach to improve cryptococcal meningoencephalitis[240]. Complement C3 is also critically involved in the recognition and clearance of *C. neoformans* in the brain[238].

Neutrophil recruitment to the CNS during aspergillosis needs further investigation. Yet, it is reported that neutrophils, recruited to the lung[242] and eye[243], mediate the clearance of

conidia[244] and restrict hyphal growth[243]. Based on the studies, it is possible that neutrophils also exert anti-*Aspergillus* activities in the brain.

5.2 | Monocytes

Monocytes, which travel through the blood to tissues where they become macrophages or dendritic cells (DCs), are crucial for controlling fungal infections in both mice and humans. (DCs were identified in the cerebrospinal fluid (CSF) of patients with severe cryptococcal meningoencephalitis[245], but limited information on brain DCs during CNS mycosis is available.) Monocytes also exert effector functions, such as phagocytosis and secretion of cytokines and chemokines[246,247]. Inflammatory monocytes express CCR2, a key chemokine receptor for monocyte migration. CCR2, together with C-X3-C Motif Chemokine Receptor 1 (CX3CR1), is critical for monocyte trafficking along inflamed vessels and subsequent accumulation in the brain[248]. Although the study is on pulmonary fungal infections, depletion of $CCR2^+$ cells in mice reduced host survival and fungal clearance in *C. albicans*[249]. The study also suggested that the antifungal activity of $CCR2^+$ cells is exerted in the first 48 hours after infection[249]. In a mouse pulmonary aspergillosis model, $CCR2^+Ly6C^+$ monocytes and their derivatives are required for antifungal $CD4^+$ T cell responses in the lung[250]. More studies describe monocytes in the CNS in *C. neoformans* infection. Monocytes are recruited and accumulate in the brain vasculature hours after systemic administration of *C. neoformans*[251]. In this context, monocytes internalize *C. neoformans* and are taken advantage of as "Trojan Horses" by the fungus to cross the BBB and enter the brain parynchema[52]. Thus, monocytes are generally antifungal, but they are used as vehicles for fungal CNS dissemination.

5.3 | T cells

The protective role of T cells, particularly $CD4^+$ T cells, is well demonstrated in fungal infections. Yet, a limited number of studies described T cells infiltrated in the CNS during fungal brain infections. Among the previous studies, *Cryptococcus* brain infection is the most well documented among all the fungal infections in the brain, probably due to the urgency to understand prevalent CNS cryptococcosis in immunocompromised individuals, such as HIV/AIDS patients with low $CD4^+$ T cell counts[252]. In comparison to $CD4^+$ T cells, $CD8^+$ T cells are rather involved in low inocula brain infection by *C. neoformans*; and $CD8^+$ T cells are relatively inefficient in clearing the fungus[253]. We also demonstrated that $CD8^+$ T cells also do not induce cryptococcal IRIS (C-IRIS), which is induced by $CD4^+$ T cells[14]. Another *ex vivo* study showed that $CD4^+$ T cells interact with microglia, as possible antigen-presenting cells, better than $CD8^+$ T cells[253]. These studies suggest a substantially less impact of $CD8^+$ T cells in CNS cryptococcosis than $CD4^+$ T cells. Nevertheless, $CD4^+$ T cell transfer into immunocompromised mice with low degree cryptococcosis did not reduce brain fungal burdens[14], implicating that brain-infiltrated $CD4^+$ T cells may also not necessarily be efficient to fight against *C. neoformans*. However, our study showed that brain-infiltrated $CD4^+$ T cells are pathogenic enough to elicit lethal outcomes in C-IRIS, with a majority of T helper cells are Th1 cells[14]. Here, IFNγ exacerbates C-IRIS[14]. Now, multiple questions remain on brain-infiltrated T cells during fungal infections. For example, which conditions make T cells in the CNS protective or pathogenic during brain fungal infection? Why are $CD8^+$ T cells less potent than $CD4^+$ T cells in providing either

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protective or detrimental outcomes in CNS fungal infections? Roles of brain-infiltrated T cells in fungal infections other than cryptococcosis are still largely elusive and merit further investigation.

5.4 | Other CNS-infiltrated leukocytes

There is limited information on CNS-infiltrated leukocytes other than those mentioned above. Natural killer (NK) cells kill *Cryptococcus* by a perforin-mediated process[254] and show intrathecal expansion and activation during *Cryptococcus* infection[245]. Brains from patients with cryptococcosis showed NK cells present within cryptococcomas[255]. B cells are also recruited to the CNS[245] and reduce *Cryptococcus* dissemination to the brain[256]. In a mouse model of invasive candidiasis, a study demonstrated a wide range of leukocytes (including NK cells, NKT cells, γδT cells, B cells, $CD4^+$ and $CD8^+$ T cells, and DCs) detected in the brain[235].

6 | HOST RESPONSES AGAINST FUNGAL PATHOGENS IN THE CNS

Immune responses in the CNS require tight and masterful regulation to limit collateral damage. Hyperactive inflammatory responses within the CNS are detrimental as they may lead to irreparable tissue damage that can leave long-term and possibly irreversible effects on the host. The CNS has historically been regarded as 'immune-privileged', isolated from the peripheral immune system by the BBB. Although a limited number of circulating immune cells are known to patrol the CNS, microglia and perivascular macrophages reside within the CNS[257] and are considered the first line of CNS defense against invading pathogens. Additionally, recent advances in neuroimmunology have presented that CNS-resident cells modulate immune responses in the CNS[258]. However, technical limitations remain in elucidating CNS-resident cells *in vivo* responses due to the difficulty in reflecting the biological functions of the cells using *ex vivo* experimental approaches. This section discusses the mechanistic and physiological outcomes of host CNS-resident cells upon detection of fungal pathogens.

6.1 | CNS immune responses against *Candida*

Although most *Candida* infections are seen in mucosal surfaces and on the skin, CNS *Candida* infections do occur, particularly in the meninges in humans. The bloodstream carries disseminated *Candida* to all organs, including the CNS. *Candida* CNS infiltration starts with traversing the BBB[259], as described in Section 3.

Once *Candida* infiltrates the brain, CNS-resident cells respond. Microglia are currently the best-studied CNS-resident cells for their responses and functions. The first article investigating microglia using a mouse model of *Candida* infection was published nearly 30 years ago. In this study, intracerebral transfer of the BV2 microglial cell line to syngeneic mice inhibited fungal growth in the brain, suggesting the overall protective role of microglia during *Candida* infection[260]. A host protective role of microglia could be attributed to their capacity to phagocytose pathogens and serve a sentinel role, by microglia promptly detecting pathogens and producing chemokines and cytokines to recruit inflammatory cells from circulation. Indeed, microglia express CXCL1 upon detection of candidalysin to recruit

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neutrophils for *Candida* clearance and release a proinflammatory cytokine IL-1β through NLRP3 inflammasome activation[261]. In microglia, the production of both CXCL1 and IL-1β depends on CARD9[261]. CXCL1 is a critical chemokine for neutrophil recruitment to efficiently clear fungus; and astrocytes assist microglial CXCL1 production by providing additional signals to microglia producing CXCL1 in response to candidalysin[261]. The involvement of astrocytes in the sentinel role of microglia is intriguing because it suggests the active participation of astrocytes at the early stage of antifungal immune responses. At this point, it is unknown if direct contact between microglia and astrocytes is required or if some soluble factors mediate the crosstalk to enhance CXCL1 production by microglia.

As described above, a major role of microglia, at least in *Candida* infection, is sensing the infection and sending a signal to recruit neutrophils in the brain. The sentinel function of microglia is reminiscent of tissue-resident macrophages in the peripheral organs, such as alveolar macrophages, which secrete chemokines CXCL1 and CXCL2 faster than other cell types during pulmonary cryptococcosis, as our lab previously reported[262]. We also demonstrated that tissue-resident macrophages produce CXCL1 and CXCL2 more efficiently than blood-borne macrophages for neutrophil recruitment upon *C. albicans* detection[263]. Here, tissue-resident macrophages are poised to induce NFκB activation and secrete CXCL1 and CXCL2 by autophagic digestion of A20 upon detecting *Candida*[263]. Indeed, microglia in naive adult mice also highly express TNF Alpha Induced Protein 3 (TNFAIP3/A20)[264]. Thus, microglia may also use autophagic A20 degradation as an approach to fulfill their role as CNS immune sentinels.

Candida infection in the CNS also promotes the accumulation of activated microglia, astrocytes, and cleaved Aβ peptide around yeast aggregates and forming fungal-induced glial granulomas[164]. Aβ has antimicrobial activities[265,266] and efficiently aids the clearance of *Candida* by enhancing microglial phagocytosis and antifungal activity[164]. However, Aβ is also the key mediator of Alzheimer's disease pathology[267]. Therefore, further investigation is warranted to understand the long-term consequence of fungal infections in the brain and their possible connection to the induction of neurodegenerative diseases in later life.

6.2 | CNS immune responses against *Cryptococcus*

Dissemination of *Cryptococcus* into the CNS can result in the development of CM[268]. Although CM was initially described over a century ago[269], immune responses of CNS-resident cells to *Cryptococcus* remain poorly understood. *Cryptococcus* forms a large polysaccharide capsule that hides components of the yeast cell wall[9], resulting in limited and delayed recognition by host immune cells[270]. Studies using BV2 microglial cells demonstrated that acapsular mutant *Cryptococcus* yeasts were more susceptible to phagocytosis and clearance than encapsulated yeasts[271]. However, both acapusular mutant and encapsulated yeasts similarly fail to elicit TNFα and IL-1β expression by BV2[271]. Interestingly, the addition of either acapusular or encapsulated yeasts to BV2 cell culture inhibits LPS-mediated TNFα expression[271]. Furthermore, *Cryptococcus* capsule components, GXM and galactoxylomannan (GalXM), also inhibit LPS-mediated TNFα expression[271], suggesting that the *Cryptococcus* capsule can inhibit TLR4 signaling, although it is not clear why acapusular mutant yeasts also inhibited the TLR4

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signaling. Here, an antibody against GXM opsonizes *C. neoformans*, enhancing microglial phagocytosis, limiting *Cryptococcus* growth[272], and inducing chemokine expression of CCL3 (MIP-1α) and CCL4 (MIP-1β)[273]. The data highlights the critical involvement of the *Cryptococcal* capsule in hindering microglia effector functions. At the same time, something other than capsule components appears to be involved in limiting cytokine expression upon detection of *C. neoformans*. Another study suggested that microglia are, unexpectedly, relatively slow to respond in a mouse model of CM[274]. Based on the flow cytometry data, seven days post-infection (dpi) was still too early for microglia to upregulate the expression of activation markers, CD11c and major histocompatibility complex class II (MHCII), and the upregulation of the two molecules was found on 14-dpi[274]. Additionally, the production of cytokines and chemokines, including TNFα, CCL3, and CCL4, required at least 21-dpi, and some molecules took more than 30-dpi for significant upregulation[274].

C. neoformans induces immune reconstitution inflammatory syndrome (IRIS), a pathological condition whereby a recovering immune system paradoxically worsens the patient's condition by excessively responding to a previously acquired *C. neoformans* infection. IRIS can also be associated with *Candida* and *Aspergillus*, but *Cryptococcus*-associated IRIS (C-IRIS) is most prevalent, and a serious concern, for HIV/AIDS patients. We used a mouse model of C-IRIS, mimicking human C-IRIS observed in immunocompromised patients, and demonstrated that the combination of previous subclinical *C. neoformans* infection and immune reconstitution by $CD4^+$ T cells led to brain swelling by dysregulation of aquaporin-4 expression in astrocytes[14]. Microglia activation was also reported in mouse C-IRIS[275]. In the mouse C-IRIS model, $CD4^+$ T, particularly Th1 cells, are involved in the lethal outcomes, but $CD8^+$ T cells are not[14]. Although it is unknown why $CD8^+$ T cells do not elicit the lethal C-IRIS outcomes, another study suggested less involvement of $CD8^+$ T cells compared to $CD4^+$ T cells in controlling *Cryptococcus* infection in the presence of microglia[253]. The study also indicated a lesser magnitude of MHC-I upregulation than MHC-II upregulation in an IFNγ-stimulated BV2 microglial cell line[253]. These studies suggest the participation of IFNγ and $CD4^+$ T cells in controlling CNS cryptococcosis, but IFNγ and $CD4^+$ T cells could cause collateral damage in the CNS, as observed in C-IRIS[14,275].

Astrocytes also undergo structural and functional changes during CNS mycosis, *i.e.,* astrogliosis. Astrogliosis is associated with destructive parenchymal cryptococcomas in humans[268]. In patients with CM, astrocytes are the primary source of CXCL10[276], one of the most highly expressed chemokines in the CSF[245]. Human astrocytoma cells have also been demonstrated to engulf cryptocci[277]. We currently do not fully understand the functions of reactive astrocytes during *in vivo Cryptococcus* infection. Yet, astrocytes may be involved in limiting CM by nitric oxide production[278] and possible $CD4^+$ T cell activation by upregulation of human leukocyte antigen (HLA) class II, as demonstrated with astrocytoma cells[277]. The involvement of neurons and oligodendrocytes in CNS cryptococcosis is not known.

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6.3 | CNS immune responses against *Aspergillus*

Although *Aspergillus* infects the brain and forms brain abscesses in immunocompromised individuals[279], limited reports are available for host cell responses in CNS aspergillosis. *A. flavus* infects the microglia cell line CHME-3 and induces expression of various cytokines, *Tlr* genes, and *Mmp* genes[280]. In particular, *Il17, Tlr7, Tlr9,* and *Mmp9* expression is highly upregulated, and the magnitude of gene expression by *A. flavus* stimulation is generally more significant than that by *C. albicans* stimulation[280]. Secreted factors from *A. fumigatus, A. flavus, A. terreus,* and *A. niger.* are toxic to primary astrocytes and an astrocyte cell line, but neurons appear to be more sensitive to *Aspergillus*-secreted toxins[281]. As demonstrated with monocytes and macrophages, conidia and hyphae of *Aspergillus* potently activate the host complement system to opsonize the fungus and support phagocytosis and killing[282]. Cerebral aspergillosis induces the expression of complement proteins (C1q, C3, and C4) by neurons, oligodendrocytes, and CNS-infiltrated myeloid cells. However, unexpectedly, microglia only showed a marginal increase in complement protein expression[283]. The complement system can enhance various proinflammatory biological functions in the brain, such as cell stimulation and induction of chemotaxis[284]. At the same time, it can lead to collateral damages in the brain with loss of neurons and oligodendrocytes[284].

7 | CONCLUSIONS

CNS mycoses are emerging as major global health concerns, causing morbidity and mortality, especially in immunocompromised individuals. Although the CNS has been deemed immune-privileged, recent data demonstrate that CNS-resident cell types, particularly glial cells, can detect and respond to invading pathogenic fungi via PRRs and initiate immune responses during infection. Also, immune cell recruitment in the CNS from the circulation during CNS fungal infections was demonstrated. Animal in vivo mycosis models on peripheral organs have provided considerable information, but our knowledge of CNS mycosis is still behind. For example, little is known about what happens to host cells and fungi in the CNS after detecting fungal invasion. Multiple factors contribute to the paucity of our knowledge in CNS mycosis, despite the unacceptable morbidity and mortality rate. The CNS environment is unique: It involves neuro-immune communications and requires tightly regulated immune responses to avoid irreparable damage. In addition, the CNS environment and structure make it technically challenging to assess host cellular responses. New technologies in studying cells in the CNS greatly merit expanding our understanding of CNS mycosis to overcome the challenges.

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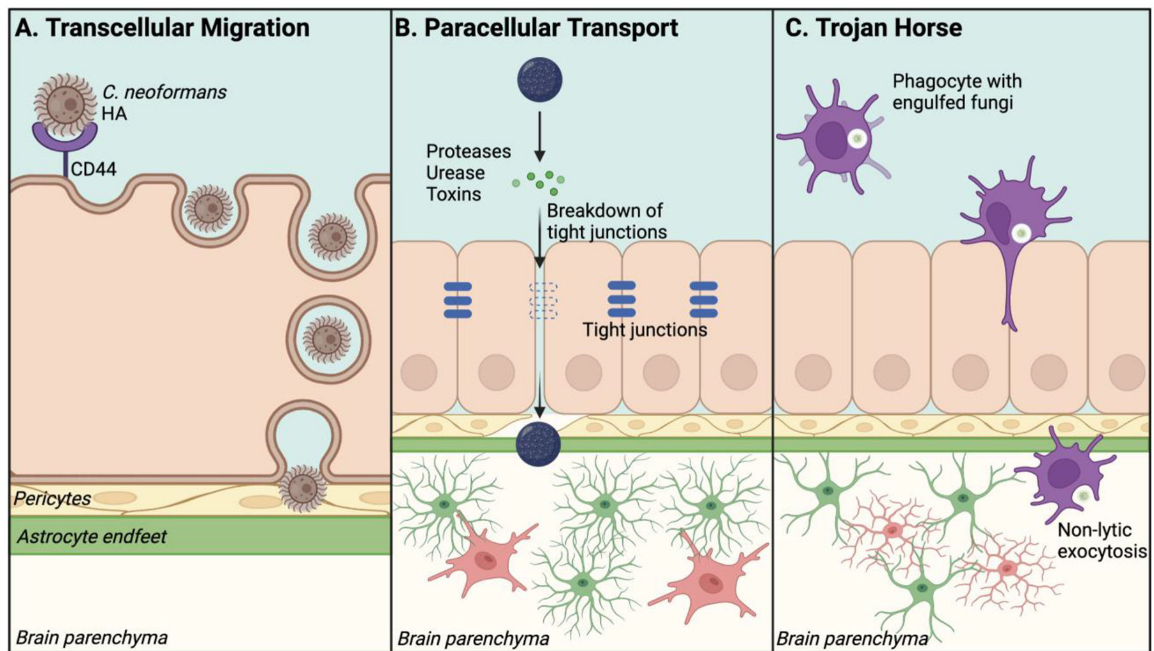

Figure 1. Mechanisms of fungal brain invasion.

(A) *Transcellular Migration*: Interaction between a fungus and host barrier cells induces endocytosis of a fungus. Then, the fungus exits from host barrier cells and crosses the BBB. Shown is an example that HA in *C. neoformans* is detected by CD44 on BMEC to initiate receptor-ligand mediated transcytosis (RMT). **(B)** *Paracellular Transport*: Molecules secreted by fungi (*e.g.*, proteases, urease) disrupt tight junctions between barrier cells to breach the BBB integrity. This allows the fungus to migrate across the endothelial layer into the brain parenchyma. **(C)** *Trojan horse*: Peripheral phagocytes uptake fungal spores or yeasts and migrate to the brain. Spores or yeasts are released from phagocytes once phagocytes migrate into the CNS. Created with BioRender.com

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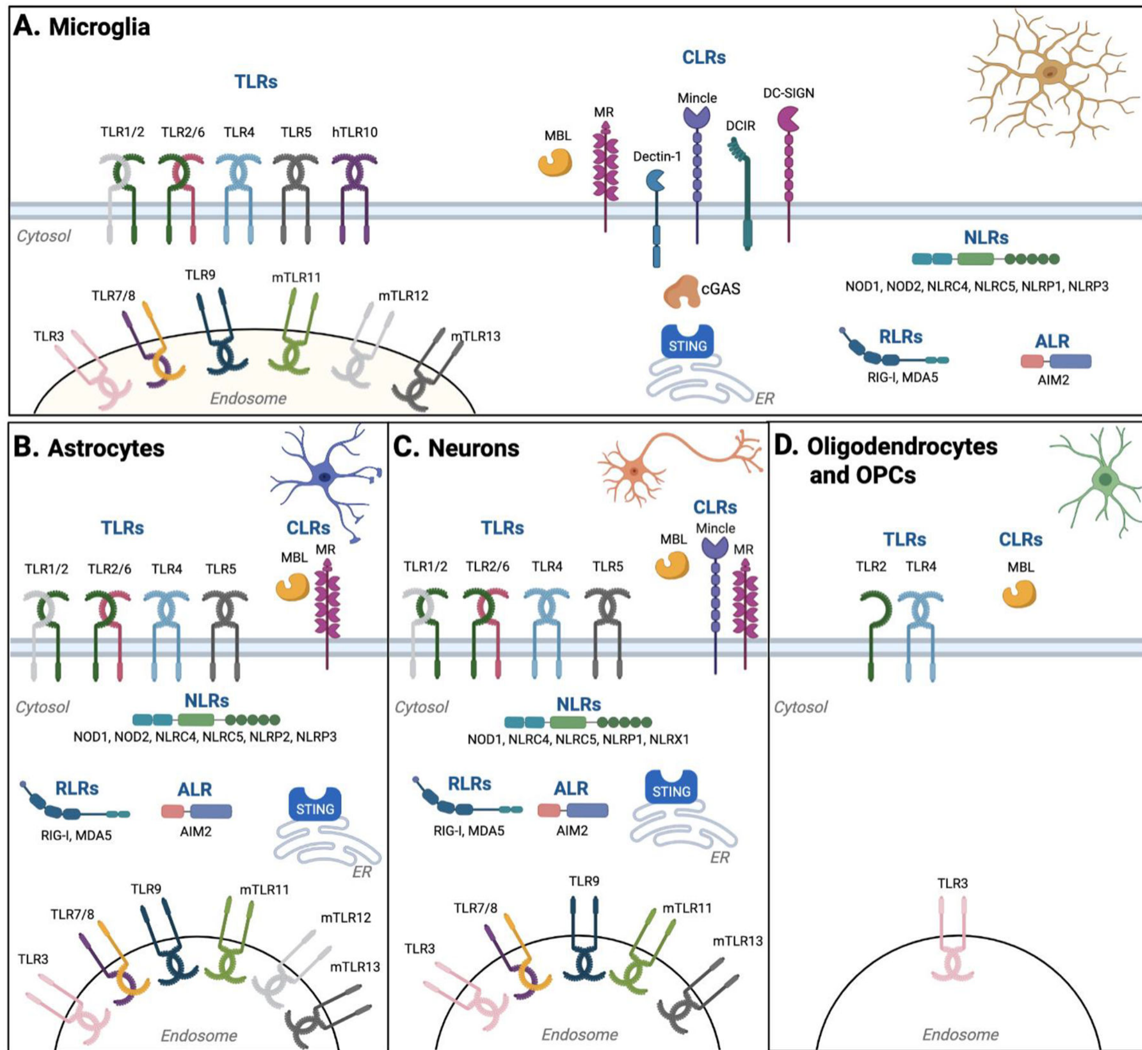

Figure 2. Pattern Recognition Receptors identified in CNS-resident cells.

CNS-resident cells express a variety of PRRs to detect DAMPs and PAMPs. **(A)** Microglia, as CNS-resident macrophage-like cells, express an extensive repertoire of PRRs, including TLRs, CLRs, NLRs, RLRs, and ALR. **(B, C)** Compared to microglia, less variety of PRRs, particularly CLRs, was reported in astrocytes (B) and neurons (C). **(D)** A limited variety of PRRs in oligodendrocytes (and oligodendrocyte progenitor cells) have been reported. Created with BioRender.com.

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Table 1.

PRR Expression in CNS-Resident Cells

	Stimulators/Ligands Described	CNS-Resident Cell	References
Toll-like Receptors (TLRs)			
TLR1 Dimerizes with TLR2	Triacyl lipopeptides α-synuclein	Microglia	72–75
		Astrocytes	75,165,166
		Neurons	202
TLR2	Peptidoglycan (PGN) Lipopeptides Lipoteichoic acid (LTA) Pam3CSK4 Zymosan	Microglia	72–75
		Astrocytes	172
		Oligodendrocytes	227,228
		Neurons	204,209,210
TLR3	poly(I:C)	Microglia	72–75,96,97
		Astrocytes	75,165,166
		Oligodendrocytes	228
		Neurons	206,211,212
TLR4	Lipopolysaccharide (LPS) Neuraminidase (NA) 4-hydroxynonenal (HNE)	Microglia	72–75,86,88,89
		Astrocytes	75,165,166,168,172
		Oligodendrocytes	229
		Neurons	204,210
TLR5	Flagellin	Microglia	72–75,106–109
		Astrocytes	75,165,166,172
		Neurons	106,211,212
TLR6 Dimerizes with TLR2	Diacyl lipopeptide	Microglia	72–75
		Astrocytes	75,165,166
		Neurons	202
TLR7	ssRNA Imidazoquinolinone R848 Imiquimod	Microglia	72–76,100,101
		Astrocytes	75,165,166,181
		Neurons	205
TLR8	ssRNA Imidazoquinolinone R848	Microglia	72–75
		Astrocytes	75,165,166
		Neurons	213
TLR9	CpG-DNA	Microglia	72–75,214
		Astrocytes	75,165,166,172,181
		Neurons	214
TLR10 (human)	Not determined	Microglia	74,75
TLR11 (murine)	Uropathogenic bacteria Profilin-like molecule from *T. gondii*	Microglia	72,73
		Astrocytes	75,165,166,184
		Neurons	184
TLR12 (murine)	Not determined	Microglia	72,73
		Astrocytes	75,165,166

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	Stimulators/Ligands Described	CNS-Resident Cell	References
TLR13 (murine)	Not determined	Microglia	72,73
		Astrocytes	75,165,166
		Neurons	73
C-type Lectin Receptors (CLRs)			
DCIR	Not determined	Microglia	285
DC-SIGN	Mannose-containing glycoproteins Fucose Myelin/MOG	Microglia	116
Mincle	SAP130 α-mannose	Microglia	286
		Neurons	215,216
Mannose Binding Lectin (MBL)	Mannose Fucose N-acetylglucosamine Amyloid β peptides	Microglia	187
		Astrocytes	187
		Oligodendrocytes	187
		Neurons	186,187
Mannose Receptor (MR)	Mannose Fucose N-acetylglucosamine	Microglia	287
		Astrocytes	186
		Neurons	186
Dectin-1	β-glucans Zymosan	Microglia	113,124,127
NOD-like Receptors (NLRs)			
NOD1	Diaminopimelic acid (DAP)	Microglia	137,138
		Astrocytes	188
		Neurons	188,217
NOD2	Muramyl dipeptide (MDP)	Microglia	137,138
		Astrocytes	188
NLRC4 (IPAF)	Palmitate	Microglia	147
		Astrocytes	146
		Neurons	219
NLRC5 (NOD4)	Not determined	Microglia	147
		Astrocytes	288
		Neurons	288
NLRX1 (NOD5)	Not determined	Neurons	221
NLRP1	Not determined	Microglia	144
		Neurons	218–220
NLRP2	ATP	Astrocytes	190
NLRP3	Lysophosphatidylcholine (LPC) Nigericin	Microglia	144–147
		Astrocytes	146
RIG-I-like Receptors (RLRs)			
RIG-I	poly(I:C)	Microglia	148
		Astrocytes	195–197
		Neurons	208,224,225

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	Stimulators/Ligands Described	CNS-Resident Cell	References
MDA5	poly(I:C)	Microglia	148
		Astrocytes	195–198
		Neurons	208,224
AIM2-like Receptors (ALRs) and other PRRs			
AIM2	Poly(dA:dT) dsDNA	Microglia	151,152,223
		Astrocytes	145,223
		Neurons	219,222,223
cGAS-STING	dsDNA	Microglia	154