What fungal CNS infections can teach us about neuroimmunology and CNS-specific immunity

Drummond, Rebecca A

DOI:
10.1016/j.smim.2023.101751

Document Version
Publisher's PDF, also known as Version of record

Citation for published version (Harvard):
Drummond, RA 2023, 'What fungal CNS infections can teach us about neuroimmunology and CNS-specific immunity', *Seminars in Immunology*, vol. 67, 101751. https://doi.org/10.1016/j.smim.2023.101751

Seminars in Immunology 67 (2023) 101751

Contents lists available at ScienceDirect

Seminars in Immunology

journal homepage: www.elsevier.com/locate/ysmim

What fungal CNS infections can teach us about neuroimmunology and CNS-specific immunity

Rebecca A. Drummond

Institute of Immunology & Immunotherapy, Institute of Microbiology and Infection, University of Birmingham, Birmingham B15 2TT, United Kingdom

ARTICLE INFO

Keywords:
Cryptococcus
Card9
Microglia
Candida
Astrocyte
Meninges
Blood-brain-barrier

ABSTRACT

Immunity to fungal infections of the central nervous system (CNS) is one of the most poorly understood subjects within the field of medical mycology. Yet, the majority of deaths from invasive fungal infections are caused by brain-tropic fungi. In recent years, there have been several significant discoveries in the regulation of neuroinflammation and the role of the immune system in tissue homeostasis within the CNS. In this review, I highlight five important advances in the neuroimmunology field over the last decade and discuss how we should capitalise on these discoveries to better understand the pathogenesis of fungal CNS infections. In addition, the latest insights into fungal invasion tactics, microglia-astrocyte crosstalk and regulation of antifungal adaptive immune responses are summarised in the context of our contemporary understanding of CNS-specific immunity.

Neuroimmunology is the relatively new field studying interactions between the immune and peripheral/central nervous systems. Several recent studies have indicated that tissues of the central nervous system (CNS) are populated with immune cells that regulate homeostatic function and are critical for neuronal development. Also, glial cells and neurons have been shown to participate and regulate immune responses both locally and systemically during sterile inflammation and infection. These advances have driven an intense interest in understanding how the immune and nervous systems converge and integrate, particularly in the context of diseases that afflict the CNS.

The CNS is prone to infection from viruses, bacteria, parasites and fungi, ranging from mild disease to life-threatening illnesses. Fungal CNS infections are typically life-threatening, partly because they infect patients with underlying vulnerabilities and there is limited treatment options for these infections. The majority of fungal CNS infections in humans are caused by *Cryptococcus neoformans*, leading to cryptococcal meningitis that is a major complication of AIDS [1]. Many other fungal species can invade the CNS, although these infections tend to be rarer and associate with specific risk factors such as inherited primary immunodeficiency CARD9 deficiency, and treatment with immune-modulating drugs (Table 1).

This review will outline five major areas of interest in the neuroimmunology field and their relevance for CNS fungal infections, highlighting recent advances in CNS-specific antifungal immunity as well as insights from non-fungal CNS infections and what we can learn from those studies.

1. Heterogeneity of tissue-resident CNS myeloid cells

The CNS is home to functionally distinct populations of tissue-resident macrophages including the meninges (meningeal macrophages), cerebral blood vessels (perivascular macrophages) and brain parenchyma (microglia) [2] (Fig. 1). Each of these populations can be further broken down into different subsets, of which our understanding is still in its infancy.

The most numerous CNS-resident macrophage is microglia [2]. These long-lived specialised cells are critical for brain development, derive from embryonic precursors and are important mediators of CNS inflammation and response to infection. Microglia are decorated with pattern recognition receptors (PRRs) that enable them to survey their environment and phagocytose pathogens and initiate downstream protective immunity. Microglia rapidly respond to the yeast *Candida albicans* via C-type lectin-dependent CARD9 signalling [3]. Microglia recognise Candidalysin toxin produced by the fungus, which activate the inflammasome in a CARD9-dependent pathway causing IL-1β release. Activated microglia then produce the chemoattractant CXCL1 to recruit neutrophils to the brain to clear infection (Fig. 1) [3]. Human CARD9 deficiency results in a failure to activate this pathway, predisposing patients to spontaneous CNS fungal infections caused by *Candida* species [4]. The organ-specific susceptibility seen in CARD9 deficiency is therefore likely attributable to microglia dysfunction, which reside in the CNS but not in other organs (e.g. the kidney) where CARD9 is redundant for neutrophil recruitment [5]. Microglia responses to other

E-mail address: r.drummond@bham.ac.uk.

https://doi.org/10.1016/j.smim.2023.101751
Received 23 November 2022;
Available online 27 March 2023

types of fungal CNS infections remain poorly defined. Early studies using microglia cell lines showed that microglia may respond to acapsular strains of *C. neoformans* but wild-type encapsulated strains elicit much poorer microglia responses [6], and as such these cells appear to be susceptible to intracellular infection similar to other macrophage populations [7,8].

In all of these studies, microglia have been examined as a whole population yet in the neurodegeneration field microglia have been shown to functionally diverge into specific activation states. For example, in mouse models of Alzheimer's disease, microglia that cluster around amyloid plaques had gene expression patterns and a lipid metabolic signature that was distinct from other microglia in the brain [9]. These plaque-associated microglia, termed 'damage-associated microglia' (DAMs), have subsequently been found in multiple CNS inflammatory diseases, including infection [10,11]. In a mouse model of chronic *C. neoformans* infection, microglia upregulated DAM markers CD11c and MHC Class II, indicating that these activation states may form in response to fungal infection as well (Fig. 1) [12]. However, other studies utilising single-cell transcriptomics and/or proteomics on microglia have revealed further subsets in the ageing brain and in response to LPS injection that are not aligned to the DAM signature [13,14]. It is therefore tempting to speculate that microglia form disease-specific subsets and/or activation states, guided by signals they receive from their environment and the pattern of PRR engagement and signalling. However, it is important to remember that while single-cell technologies have significantly advanced our understanding of immune cell heterogeneity, they do not always reveal the existence of new subsets. For example, immune cells transitioning between activation states may appear as distinct clusters within datasets [13]. That is particularly important to consider when studying myeloid cells which are inherently plastic in their polarisation and responses to inflammation. Although single-cell technologies provide in-depth information about heterogeneous populations, more effort should be taken to compare data across multiple datasets to look for similarities between different infection and disease models, as this could lead to better understanding of common triggers for microglia activation states.

In addition to microglia, there are several populations of CNS-resident border macrophages that populate the meninges, choroid plexus and perivascular spaces. During infection, these populations can be replenished and/or expanded by infiltrating monocytes that differentiate into inflammatory macrophages after CNS entry [2]. How these cells function in the context of invasive fungal infection is largely unknown, but there is some evidence from other fields that microglia and border macrophages exhibit divergent roles during infection. In mice infected with the parasite *Toxoplasma gondii*, microglia rapidly produced the cytokine IL-1α that was required for infection control [15]. In contrast, macrophages produced IL-1β. Parasite infection was uncontrolled in mice lacking IL-1R1, and this was replicated in mice lacking IL-1α but not IL-1β, pinpointing microglia as the main regulators of protective anti-parasite immunity in the brain [15]. This study also revealed that microglia and macrophages are differentially equipped to deal with infection, and have functionally distinct roles in infection control. Whether such divergence occurs in CNS fungal infection has not been formally shown, although early work on CNS macrophage responses to *C. neoformans* indicated that the monocyte-derived border macrophages may be critical for activating anti-fungal CD4 T-cell responses in the brain whereas microglia were not capable of this [16]. This was done by generating bone marrow chimeras where the radio-resistant microglia population expressed MHCII and the radio-sensitive border macrophages were MHCII-deficient. In those mice, adoptive transfer of pre-activated CD4 T-cells failed to lower fungal burdens in the brain compared to mice that expressed MHCII within the border macrophage compartment or both microglia and border macrophages [16]. In line with a more prominent role for border macrophages in the protection against *C. neoformans* infections, recent work in mice demonstrated that *C. neoformans* was largely localised to perivascular spaces and infected macrophages that resided there [8], indicating that there may be greater uptake and processing of antigen at the perivascular spaces than in the parenchyma where microglia reside. Future work will need to carefully delineate the different populations of myeloid cells in the brain and determine the relative dependence on each for control of fungal infection, with particular attention paid to the heterogeneity observed in these populations and how they may be remodelled during infection.

2. Meningeal immunity and inflammation

The meninges is a complex layered tissue populated with specialised macrophages, monocytes and lymphocytes (Fig. 1). Single-cell RNA sequencing of human meninges showed that macrophages and structural cells undergo functional specialisation in the different meningeal layers and that disease and age profoundly affect these responses within the microenvironmental niches of this tissue [17,18]. Moreover, recent exciting studies have demonstrated that the meninges can act as a gateway for immune cells to enter the brain from the skull bone marrow providing a source of inflammatory leukocytes to help fight infection

Table 1
Characteristics of main causes of fungal CNS infections in humans. The major human fungal pathogens that have been described to cause CNS infection are listed alongside their relative occurrence (within total fungal CNS infections in humans), main risk factors, known CNS invasion tactics and key protective immune pathways that have been shown in vivo. Many other fungal species may also cause sporadic cases of CNS infection following uncontrolled disseminated infection (e.g. *Pneumocystis*, some dematiaceous fungi) however outside of general iatrogenic immunosuppression, there is little understanding of the risk factors and immune responses required to protect against these infections.

Fungal Species	Occurrence of total fungal CNS infections	Risk Factors	CNS invasion tactics	CNS protective immune pathways	Selected References
Cryptococcus neoformans	High	Chronic HIV infection/AIDS, CD4 lymphocytopenia, Iatrogenic immunosuppression, Ibrutinib therapy	Proliferation within cerebral blood vessels, urease-dependent invasion, "Trojan Horse" delivery within patrolling monocytes	IFNγ-mediated activation of macrophages	[1]
Candida albicans	Low	CARD9 deficiency, Iatrogenic immunosuppression	Hyphae formation (Als3 expression)	Neutrophil recruitment, CARD9-dependent activation of microglia	[4,53]
Aspergillus fumigatus	Low	Chronic lung disease/infection, Iatrogenic immunosuppression, Ibrutinib therapy	Not known	Not known	[52]
Histoplasma capsulatum	Low	HIV infection, Organ transplant, Corticosteroids	Not known	Not known	[54]
Blastomyces dermatitidis	Low	Organ transplant, HIV infection	Not known	Not known	[55]

locally [19–21].

Our understanding of antifungal immune responses in the meninges is currently very limited. A characterisation of myeloid cell responses to *C. neoformans* was published by the Sorrell lab, which revealed a large infiltration of monocytes into the infected perivascular spaces [8]. That is important because recent work on the ontogeny of meningeal macrophages during viral encephalitis showed that these immune cells are sensitive to extensive remodelling following monocyte infiltration, with potential long-term consequences. Rua and colleagues showed that lymphocytic choriomeningitis virus (LCMV) infection caused a depletion of meningeal macrophages which were subsequently replenished by monocyte-derived macrophages in a process that required IFNγ [22]. Those replenished macrophages remained in the meninges long-term but were impaired in their ability to quench subsequent neuroinflammation and initiate repair responses [22]. These data indicate that remodelling of CNS-resident macrophages occurs as a result of infection, and could potentially influence responses to subsequent insults and injury. Whether such remodelling occurs during fungal infection is not known, but it is an interesting point to consider in light of work that demonstrates how fungi and fungal cell wall components can elicit long-lasting epigenetic changes in macrophages [23].

In recent years, several important discoveries about meningeal anatomy with relevance for inflammation and immunity have been discovered. First, a lymphatic system that drains antigen from the CNS was found within the meninges with subsequent demonstrations of CNS-resident antigen-presenting cells (APCs) interacting with T-cells in the dural sinuses to regulate adaptive immune responses [24] (see Section 4). Second, several groups recently observed and described channels in the skull adjacent to the meninges [19–21]. In mouse models of stroke, neutrophils in the inflamed meninges originated from skull bone

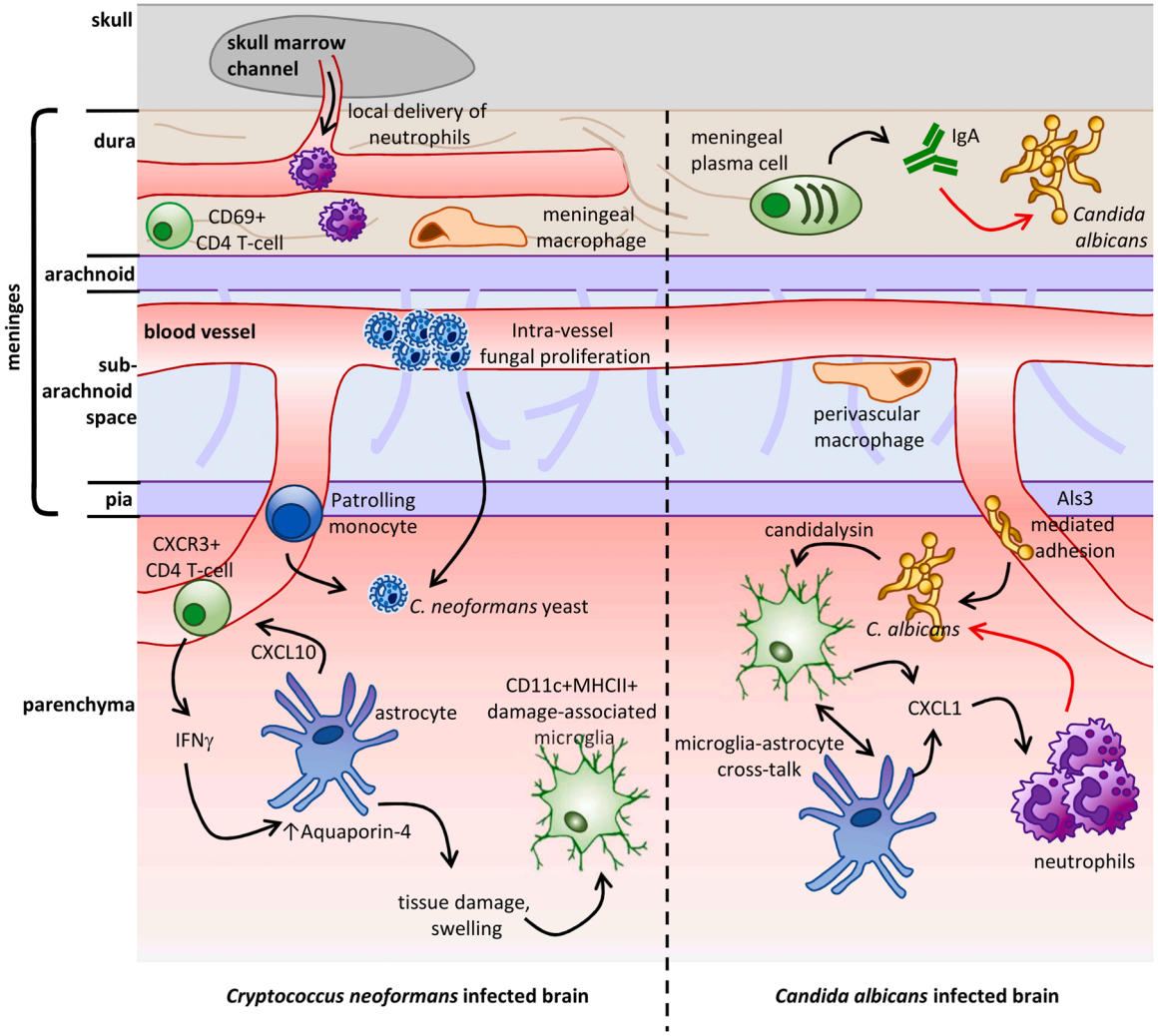

Fig. 1. Overview of CNS-specific antifungal immunity. The meninges sit directly below the skull and are composed of several layers (dura, arachnoid, sub-arachnoid space, pia). The first layer is called the dura and is where the majority of immune cells (e.g. meningeal macrophages, CD69 + resident T-cells, plasma cells) reside. Blood vessels and lymphatics run through the dura. Channels connect the dura to skull bone marrow where localised delivery of immune cells (e.g. neutrophils) occurs during neuroinflammation. Below the dura, the sub-arachnoid space sits between the arachnoid and pia layers. Blood vessels run through the sub-arachnoid space and connect with the parenchyma of the brain. *On the left side of the diagram*, fungal invasion tactics and immune pathways activated during *Cryptococcus neoformans* infection is shown. *C. neoformans* yeast can proliferate within blood vessels leading to tension and brain dissemination, or alternatively travel to the brain within infected patrolling monocytes. CD4 T-cells enter the brain via CXCR3-CXCL10 axis initiated by astrocytes. Cross-talk between astrocytes and IFNγ-producing T-cells leads to tissue damage and swelling via aquaporin-4 expression, which may drive formation of damage-associated microglia. *The right side of the diagram* focuses on *Candida albicans* brain infection, with defined anti-fungal killing mechanisms depicted with red arrows. In the meninges, *Candida* invasion is limited by IgA-producing meningeal plasma cells. *C. albicans* invades the parenchyma following adhesion to blood vessels via Als3, and produces the toxin Candidalysin once in the tissue. That elicits a CARD9-dependent activation of microglia and downstream crosstalk with astrocytes leading to CXCL1 production and recruitment of neutrophils which clear and kill the fungus.

marrow and not from distant sites such as the tibia, trafficking into the meninges via these skull bone channels which connected the bone marrow niche to the meninges (Fig. 1) [19]. The authors theorised that this mechanism allows rapid local delivery of short-lived myeloid cells to the meninges. Several fungal infections, particularly *C. albicans*, cause an emergency granulopoeisis response in bone marrow characterised by a large influx of neutrophils into the blood and infected tissues [25]. During CNS infection, neutrophils also rapidly accumulate in the *C. albicans*-infected brain [5,26] but their origin and involvement in meningeal inflammation remains unknown. Future studies will need to account for these recent advances in our understanding of meninges anatomy when analysing immunity to fungal CNS infections. The majority of studies have so far focused on brain tissue with little focus on the meninges. Yet, it is becoming clear that both immune cells and pathogens use the meninges as an entry point into the CNS and that localised control of CNS-specific immune responses occur in this tissue.

3. Fungal CNS invasion mechanisms and blood vessel integrity

The mechanisms by which fungi gain entry to the CNS are not fully elucidated although key virulence factors involved in invasion for *Candida* and *Cryptococcus* species have been defined (Fig. 1). *C. albicans* likely enters the CNS by invading and damaging the blood-brain-barrier (BBB), a process that was thought to require hyphae since hyphal-expressed protein Als3 has been shown to mediate brain infection by this fungus as well binding to brain endothelial cells that make up the BBB [27]. However, yeast-locked mutants are also able to infect the brain and actually cause a greater level of infection in this tissue than hyphal-forming strains [3]. This is because while hyphal factors may promote invasion of the CNS, hyphae also produce Candidalysin toxin that elicits strong pro-inflammatory responses from CNS-resident microglia leading to fungal clearance [3]. For *C. neoformans*, multiple mechanisms of CNS entry have been identified including paracellular invasion by 'free' yeast across the BBB [28], and intracellular residence within infiltrating monocytes in a mechanism commonly referred to as 'Trojan Horse' [29] (Fig. 1). Intravital imaging studies in mice and zebrafish showed that extracellular *C. neoformans* yeast cells stop inside blood vessels due to physical constraints on their size and width of the blood vessel, and from this point the yeast either invade the blood vessel endothelium in a urease-dependent mechanism [28], or proliferate within the blood vessel causing tension and rupture [30]. A combination of intravital and careful adoptive transfer studies have shown that *C. neoformans* may also invade the CNS within infected monocytes [29,31]. Patrolling monocytes were shown to be the main subset mediating this mode of fungal invasion in an intravenous model of *C. neoformans* infection, and disrupting monocyte binding to brain endothelium by blocking VCAM1/VLA4 interactions reduced the infiltration of these cells to the CNS [31].

In all of these studies on fungal invasion tactics, understanding blood vessel integrity and the molecular interactions mediating binding of fungi and/or infected host cells to the vasculature is critical. Close examination of cerebral and meningeal blood vessels during fungal CNS infection has the potential to reveal how fungi invade the CNS and the resulting damage caused to CNS tissues by infection. Recent studies have shown that blood vessel integrity in the CNS is tightly regulated by tissue-resident macrophages and microglia. A small specialised population of microglia termed 'capillary-associated microglia' (CAMs), were found to reside alongside blood vessels and regulate vasodilation via P2RY12 signalling [32]. Depletion of microglia or genetic deletion of *P2ry12* in mice resulted in altered blood flow dynamics to the brain [32]. Indeed, a different study reached similar conclusions in a model of sterile inflammation, where the authors observed microglia recruitment to inflamed cerebral blood vessels in a CCR5-dependent manner [33]. When inflammation was sustained, microglia became pathological and phagocytosed components of the BBB contributing to loss of integrity and increased permeability [33]. In a mouse model of traumatic brain injury, monocytes accumulated in the meninges and developed into macrophages with wound-healing signatures to repair blood vessels and promote angiogenesis [34], demonstrating that meningeal macrophages also have a critical role in maintaining blood vessel integrity.

Increased permeability of the BBB has been observed in multiple animal and in vitro models of invasive fungal infection [35–37], but the specific events leading to this are not fully defined. Recent work by the Johnston lab used live cell imaging in zebrafish to demonstrate that *C. neoformans* caused blood vessel rupture, which likely contributes towards its CNS dissemination and BBB dysfunction [30]. While many studies in the fungal immunology field have focused on in vitro models for the BBB and identifying fungal virulence factors mediating invasion, it is worth noting that by studying how blood vessels are affected and repaired during infection we may be able to identify the cells and molecular interactions promoting fungal dissemination to the CNS, both directly and indirectly.

4. Activation of adaptive immunity in the CNS

The CNS has an extensive lymphatic system and tissue-resident lymphocytes that have been shown to be important mediators of tissue homeostasis. For example, a population of CD69 + CD4 + T-cells reside in the mouse and human brain and are required for microglia maturation and transition to the 'adult' stage of development [38]. These brain-resident CD4 T-cells are permitted entry to the CNS following activation in the periphery, a process that is shaped by the intestinal microbiome [38]. CNS lymphatic vessels drain antigen from the brain and meninges into the cervical lymph nodes. Sophisticated microscopy studies by the Kipnis lab showed that CNS-derived antigens are captured by APCs in the dural sinuses, which presented the antigen to local CD4 T-cells under the steady-state [24]. CNS-resident myeloid cells also participate in activating CD4 and CD8 T-cells during infection, with studies demonstrating a critical role for both microglia and meningeal macrophages in activating anti-viral T-cells [22,39].

Regulation of antifungal T-cell responses in the CNS has largely focused on *C. neoformans* infections. This is because CD4 T-cells are critical for protection against this infection [1], but can also mediate significant tissue damage and inappropriate inflammation thus contributing towards meningitis [12]. The majority of risk factors that predispose to *C. neoformans* infections in humans either affect CD4 T-cell number or function, or interrupt the crosstalk between CD4 T-cells and macrophages (Table 1). There is therefore great interest in understanding how CD4 T-cells provide protection against this disease by identifying the signals they provide to macrophages to activate fungal killing, such as IFNγ. Indeed, IFNγ immunotherapy appeared to be protective in humans with HIV-associated cryptococcal meningitis [40], presumably by boosting antifungal activity of macrophages within the CNS although this has not been formally tested. While IFNγ-producing CD4 T-cells are needed for fungal clearance, these lymphocytes have also been shown to mount inappropriate inflammatory responses in the CNS in patients with *C. neoformans* infections. Patients receiving anti-retroviral therapy (ART) as treatment for chronic HIV infection can subsequently develop immune reconstitution inflammatory syndrome (IRIS), a condition characterised by pro-inflammatory responses to an undiagnosed or previously treated *C. neoformans* infection as CD4 T-cell numbers recover [41]. Work by the Olszewski lab demonstrated that CD4 T-cells were the main driver of tissue damage and disease in late-stage chronic *C. neoformans* infection, in a mouse model of post-infectious IRIS [12]. While mice depleted of CD4 T-cells were unable to clear *C. neoformans* brain infection, those mice survived longer than their T-cell-replete counterparts. The authors demonstrated that this was because CD4 T-cell depletion alleviated meningitis symptoms and tissue damage in the late stages of disease [12]. Further work by the Shinohara lab showed that IFNγ was a primary mediator of these pathological responses by CD4 T-cells in the brain [42]. Using a novel animal model of IRIS with highly virulent serotype A *C. neoformans*, the

Shinohara lab showed that an influx of IFNγ-producing CD4 T-cells were responsible for brain inflammation and swelling by upregulating aquaporin-4 expression on astrocytes. Aquaporin-4 is a molecule involved with water regulation in the brain [42] (Fig. 1). Pathologic recruitment of CD4 T-cells to the fungal-infected brain is mediated by the chemokine CXCL10, which binds to the receptor CXCR3 on IFNγ+ CD4 T-cells [43]. CXCL10 was readily detectable in the CSF of cryptococcal meningitis patients, and is one of the most highly produced chemokines during experimental *C. neoformans* infection [43]. Mice deficient in CXCR3 were protected from T-cell-mediated immunopathology, without affecting fungal brain burdens. Although CXCL10 is mostly produced by myeloid cells, surprisingly the major cellular source of CXCL10 in the chronically-infected *C. neoformans* brain was astrocytes while microglia were largely negative for this chemokine [43] (Fig. 1). These results point to several outstanding questions about how astrocytes are involved with the immunopathology of fungal CNS infections (see Section 5).

Humoral immunity, mediated by B-cells, is the other major arm of adaptive immune responses. B-cells producing antibody (plasma cells) are important for antifungal immune responses because antifungal antibodies can opsonise fungal cells to boost phagocytosis by macrophages, and activate other mechanisms of protective immunity including complement. Antibodies can be produced in different functional classes (IgG, IgD, IgM, IgA, IgE) which determines their receptor binding and tissue localisation. In the meninges, the mucosal associated IgA subtype appears to be the dominant functional class secreted by meningeal-residing plasma cells [44]. Interestingly, plasma cells in the meninges are educated by signals deriving from the gut microbiome, since they remained clonally related to intestinal B-cells and germ-free mice lacked these populations [44]. During *C. albicans* CNS invasion, meningeal B-cells increased their production of IgA which acted to trap invading yeast cells within the dural sinuses (Fig. 1). In mice lacking IgA or specifically depleted of meningeal plasma cells, *C. albicans* caused a greater infection of the brain tissue and increased mortality [44]. Therefore, local lymphocytes residing in the meninges can provide tissue-specific protection against fungal pathogens to limit their invasion and subsequent disease. In addition to demonstrating the critical role of meningeal plasma cells to barrier protection, this study also revealed that the meninges is a major *C. albicans* entry point to the CNS, thus reinforcing the call for future studies to examine this complex barrier tissue in more detail when determining fungal invasion tactics in the CNS.

5. Pathways mediating astrocyte antimicrobial immunity

Astrocytes are an abundant glial cell population in the CNS that carry out numerous homeostatic functions including provision of trophic support, protection and envelopment of neuronal synapses and they form a major component of the BBB. In recent years, there has been an intense interest in how astrocytes respond and contribute to neuroinflammation and immune responses within the CNS. In several neurodegenerative disorders (e.g. Alzheimer's, Parkinson's, Multiple Sclerosis), astrocytes take on an inflammatory phenotype characterised by enhanced expression of complement proteins and glial-acidic fibrillary protein (GFAP) that is termed A1 [45,46]. In contrast, A2 astrocytes upregulate expression of trophic factors for neurons and appear to be involved with tissue repair and important for resolution phases following an inflammatory event [45]. Both A1 and A2 activation states have now been described in several mouse models and human diseases, including infection. The main driver of A1 activation in astrocytes is inflammatory crosstalk with microglia [45]. In response to tissue damage and/or PRR signalling, microglia produce inflammatory cytokines and increase production of C1q. This in turn drives an A1 functional phenotype, which is neurotoxic and loses many of the normal homeostatic functions of healthy astrocytes [45].

Microglia-astrocyte crosstalk was found to be a critical step in the protective immune response against *C. albicans* brain infection (Fig. 1). Neutrophil recruitment to the fungal-infected brain required CXCL1 which was produced by microglia. However, in vitro experiments revealed that microglia were unable to produce this chemokine without the presence of astrocytes [3], demonstrating that astrocyte-microglia crosstalk was necessary for initiating protective neuroinflammation in *C. albicans* infection. Microglia-astrocyte crosstalk has also been identified as an important mediator of immunity to meningitis-causing bacteria. In a model of neonatal bacterial meningitis, microglia produced TNFα in response to bacterial flagellin, which in turn stimulated C3 production from astrocytes to drive neuroinflammation [47]. Interestingly, bacteria were found to dampen their expression of flagellin in the CNS which helped promote bacterial survival and prolong infection. Flagellin expression could be induced by threonine, which is found in low levels within the CNS thus explaining why CNS-infiltrating bacteria had lower flagellin levels than bacteria infecting other organs [47].

In addition to microglia, astrocytes can be shaped and influenced by leukocytes that infiltrate the CNS during infection. For example, in a mouse model of cryptococcal IRIS, infiltrating CD4 T-cells were found to mediate tissue damage via production of IFNγ. *In vitro* experiments showed that IFNγ stimulation of cultured astrocytes could cause direct upregulation of aquaporin-4, indicating that astrocytes may respond directly to IFNγ made by pathologic T-cells and become involved with tissue swelling and fluid dysregulation in the brain during this disease [42]. In parasitic infections, astrocytes have been shown to directly respond to either parasites or cytokines to mediate neuroinflammation and protection against infection. Astrocytes have been shown to uptake and accumulate extracellular vesicles from *Plasmodium* parasites, which led to upregulation of immune signalling pathways similar to what has been described for A1 astrocytes [48]. In *Toxoplasma gondii* infection, astrocytes were found to produce the alarmin IL-33 during infection, which acted in an autocrine manner to stimulate production of chemokines that recruited Th1 T-cells and iNOS-producing monocytes to the CNS [49]. Loss of IL-33 signalling specifically on astrocytes led to uncontrolled parasite burden in the CNS, whereas loss of IL-33 signalling in microglia/macrophages had no effect [49].

Taken together, there is clear evidence that astrocytes form an important arm of CNS-specific immune responses. Yet, we know very little about how these glial cells participate in antimicrobial immune responses and this is especially true for fungi. It is not understood if these cells directly recognise or phagocytose fungi in vivo, or whether their antifungal functions are dependent on crosstalk with microglia and/or infiltrating inflammatory cells. In the neurodegeneration field, significant advances have been made in understanding pathology by characterising the kinetics and development of different astrocyte activation states and have indicated that these cells could be a fresh target for therapy. Therefore, further study of astrocytes and their responses during fungal CNS infections is warranted, particularly in light of recent work that has indicated these cells may drive damaging pathology in the CNS and limit resolution and repair pathways.

6. Final remarks

The pathology of fungal CNS infections remains one of the most poorly understood areas within the medical mycology field. While this review has focused on CNS-specific responses to *Candida* and *Cryptococcus*, it is important to note that many other fungi cause CNS infection in humans but our understanding of them is critically low. CNS aspergillosis, for example, is a lethal infection that has been increasingly reported in patients treated with cancer drug ibrutinib [50,51], which impairs anti-*Aspergillus* immune responses in the lung and increases the risk of developing systemic infection [52]. The number of patients developing life-threatening fungal infections is set to increase is the next few years, in part by enhanced application of immune modulating drugs in the clinic. Studies focused on CNS-specific immune responses have typically lagged behind other tissues, in part because of difficulty in

accessing this tissue. Moreover, there is an additional need for stringent protocol development to handle and isolate CNS-resident cells, which are highly sensitive to changes in their environment and can rapidly change their functional phenotype during tissue digestion. However, advent of new technologies and increased uptake of advanced imaging techniques have enabled the development of fundamental new insights into how immune responses initiate and resolve within the CNS. For example, the use of single-cell sequencing approaches and intravital imaging have enabled in depth analysis of rare leukocytes in meninges, and have led to the discovery of new activation states and anatomical structures important for CNS localised immune responses. Increased uptake of these approaches in the fungal immunology field will be important to develop an understanding of fungal CNS invasion and regulation of CNS-specific antifungal immunity, which is critically needed to develop new therapeutic strategies for these diseases.

Acknowledgements

This work was supported by funding from the Medical Research Council (MR/S024611) and Academy of Medical Sciences (SBF004_1008).

References

[1] S.H. Mohamed, T.K. Nyazika, K. Ssebambulidde, M.S. Lionakis, D.B. Meya, R. A. Drummond, Fungal CNS infections in Africa: the neuroimmunology of cryptococcal meningitis, Front. Immunol. 13 (2022).
[2] M. Prinz, D. Erny, N. Hagemeyer, Ontogeny and homeostasis of CNS myeloid cells, Nat. Immunol. 18 (4) (2017) 385–392.
[3] R.A. Drummond, M. Swamydas, V. Oikonomou, B. Zhai, I.M. Dambuza, B. C. Schaefer, A.C. Bohrer, K.D. Mayer-Barber, S.A. Lira, Y. Iwakura, S.G. Filler, G. D. Brown, B. Hube, J.R. Naglik, T.M. Hohl, M.S. Lionakis, CARD9+ microglia promote antifungal immunity via IL-1β- and CXCL1-mediated neutrophil recruitment, Nat. Immunol. 20 (5) (2019) 559–570.
[4] R.A. Drummond, L.M. Franco, M.S. Lionakis, Human CARD9: a critical molecule of fungal immune surveillance, Front. Immunol. 9 (2018) 1836.
[5] R.A. Drummond, A.L. Collar, M. Swamydas, C.A. Rodriguez, J.K. Lim, L. M. Mendez, D.L. Fink, A.P. Hsu, B. Zhai, H. Karauzum, C.M. Mikelis, S.R. Rose, E. M.N. Ferre, L. Yockey, K. Lemberg, H.S. Kuehn, S.D. Rosenzweig, X. Lin, P. Chittiboina, S.K. Datta, T.H. Belhorn, E.T. Weimer, M.L. Hernandez, T.M. Hohl, D.B. Kuhns, M.S. Lionakis, CARD9-dependent neutrophil recruitment protects against fungal invasion of the central nervous system, PLoS Pathog. 11 (12) (2015), e1005293.
[6] R. Barluzzi, A. Brozzetti, D. Delfino, F. Bistoni, E. Blasi, Role of the capsule in microglial cell-Cryptococcus neoformans interaction: impairment of antifungal activity but not of secretory functions, Med. Mycol. 36 (4) (1998) 189–197.
[7] S.C. Lee, D.W. Dickson, A. Casadevall, Pathology of cryptococcal meningoencephalitis: Analysis of 27 patients with pathogenetic implications, Hum. Pathol. 27 (8) (1996) 839–847.
[8] K. Kaufman-Francis, J.T. Djordjevic, P.G. Juillard, S. Lev, D. Desmarini, G.E. R. Grau, T.C. Sorrell, The early innate immune response to, and phagocyte-dependent entry of, cryptococcus neoformans map to the perivascular space of cortical post-capillary venules in neurocryptococcosis, Am. J. Pathol. 188 (7) (2018) 1653–1665.
[9] H. Keren-Shaul, A. Spinrad, A. Weiner, O. Matcovitch-Natan, R. Dvir-Szternfeld, T. K. Ulland, E. David, K. Baruch, D. Lara-Astaiso, B. Toth, S. Itzkovitz, M. Colonna, M. Schwartz, I. Amit, A unique microglia type associated with restricting development of Alzheimer's disease, Cell 169 (7) (2017) 1276–1290, e17.
[10] S.F. Farhadian, S.S. Mehta, C. Zografou, K. Robertson, R.W. Price, J. Pappalardo, J. Chiarella, D.A. Hafler, S.S. Spudich, Single-cell RNA sequencing reveals microglia-like cells in cerebrospinal fluid during virologically suppressed HIV, JCI Insight 3 (18) (2018).
[11] A. Deczkowska, H. Keren-Shaul, A. Weiner, M. Colonna, M. Schwartz, I. Amit, Disease-Associated microglia: a universal immune sensor of neurodegeneration, Cell 173 (5) (2018) 1073–1081.
[12] L.M. Neal, E. Xing, J. Xu, J.L. Kolbe, J.J. Osterholzer, B.M. Segal, P.R. Williamson, M.A. Olszewski, CD4+ T cells orchestrate lethal immune pathology despite fungal clearance during cryptococcus neoformans meningoencephalitis, mBio 8 (6) (2017) e01415–17.
[13] T.R. Hammond, C. Dufort, L. Dissing-Olesen, S. Giera, A. Young, A. Wysoker, A. J. Walker, F. Gergits, M. Segel, J. Nemesh, S.E. Marsh, A. Saunders, E. Macosko, F. Ginhoux, J. Chen, R.J.M. Franklin, X. Piao, S.A. McCarroll, B. Stevens, Single-Cell RNA sequencing of microglia throughout the mouse lifespan and in the injured brain reveals complex cell-state changes, Immunity 50 (1) (2019) 253–271, e6.
[14] C. Sousa, A. Golebiewska, S.K. Poovathingal, T. Kaoma, Y. Pires-Afonso, S. Martina, D. Coowar, F. Azuaje, A. Skupin, R. Balling, K. Biber, S.P. Niclou, A. Michelucci, Single-cell transcriptomics reveals distinct inflammation-induced microglia signatures, EMBO Rep. 19 (11) (2018), e46171.
[15] S.J. Batista, K.M. Still, D. Johanson, J.A. Thompson, C.A. O'Brien, J.R. Lukens, T. H. Harris, Gasdermin-D-dependent IL-1α release from microglia promotes protective immunity during chronic Toxoplasma gondii infection, Nat. Commun. 11 (1) (2020) 3687.
[16] K. Aguirre, S. Miller, MHC class II-positive perivascular microglial cells mediate resistance to Cryptococcus neoformans brain infection, Glia 39 (2) (2002) 184–188.
[17] A. Zelco, V. Börjesson, J.K. de Kanter, C. Lebrero-Fernandez, V.M. Lauschke, E. Rocha-Ferreira, G. Nilsson, S. Nair, P. Svedin, M. Bemark, H. Hagberg, C. Mallard, F.C.P. Holstege, X. Wang, Single-cell atlas reveals meningeal leukocyte heterogeneity in the developing mouse brain, Genes Dev. 35 (15–16) (2021) 1190–1207.
[18] A.Z. Wang, J.A. Bowman-Kirigin, R. Desai, L.-I. Kang, P.R. Patel, B. Patel, S. M. Khan, D. Bender, M.C. Marlin, J. Liu, J.W. Osbun, E.C. Leuthardt, M. R. Chicoine, R.G. Dacey, G.J. Zipfel, A.H. Kim, D.G. DeNardo, A.A. Petti, G. P. Dunn, Single-cell profiling of human dura and meningioma reveals cellular meningeal landscape and insights into meningioma immune response, Genome Med. 14 (1) (2022) 49.
[19] F. Herisson, V. Frodermann, G. Courties, D. Rohde, Y. Sun, K. Vandoorne, G. R. Wojtkiewicz, G.S. Masson, C. Vinegoni, J. Kim, D.-E. Kim, R. Weissleder, F. K. Swirski, M.A. Moskowitz, M. Nahrendorf, Direct vascular channels connect skull bone marrow and the brain surface enabling myeloid cell migration, Nat. Neurosci. 21 (9) (2018) 1209–1217.
[20] A. Cugurra, T. Mamuladze, J. Rustenhoven, T. Dykstra, G. Beroshvili, Z. J. Greenberg, W. Baker, Z. Papadopoulos, A. Drieu, S. Blackburn, M. Kanamori, S. Brioschi, J. Herz, L.G. Schuettpelz, M. Colonna, I. Smirnov, J. Kipnis, Skull and vertebral bone marrow are myeloid cell reservoirs for the meninges and CNS parenchyma, Science 373 (6553) (2021) eabf7844.
[21] S. Brioschi, W.-L. Wang, V. Peng, M. Wang, I. Shchukina, Z.J. Greenberg, J. K. Bando, N. Jaeger, R.S. Czepielewski, A. Swain, D.A. Mogilenko, W.L. Beatty, P. Bayguinov, J.A.J. Fitzpatrick, L.G. Schuettpelz, C.C. Fronick, I. Smirnov, J. Kipnis, V.S. Shapiro, G.F. Wu, S. Gilfillan, M. Cella, M.N. Artyomov, S. H. Kleinstein, M. Colonna, Heterogeneity of meningeal B cells reveals a lymphopoietic niche at the CNS borders, Science 373 (6553) (2021) eabf9277.
[22] R. Rua, J.Y. Lee, A.B. Silva, I.S. Swafford, D. Maric, K.R. Johnson, D.B. McGavern, Infection drives meningeal engraftment by inflammatory monocytes that impairs CNS immunity, Nat. Immunol. 20 (4) (2019) 407–419.
[23] J. Quintin, Fungal mediated innate immune memory, what have we learned? Semin. Cell Dev. Biol. 89 (2019) 71–77.
[24] J. Rustenhoven, A. Drieu, T. Mamuladze, K.A. de Lima, T. Dykstra, M. Wall, Z. Papadopoulos, M. Kanamori, A.F. Salvador, W. Baker, M. Lemieux, S. Da Mesquita, A. Cugurra, J. Fitzpatrick, S. Sviben, R. Kossina, P. Bayguinov, R. R. Townsend, Q. Zhang, P. Erdmann-Gilmore, I. Smirnov, M.-B. Lopes, J. Herz, J. Kipnis, Functional characterization of the dural sinuses as a neuroimmune interface, Cell 184 (4) (2021) 1000–1016, e27.
[25] H. Hirai, P. Zhang, T. Dayaram, C.J. Hetherington, S. Mizuno, J. Imanishi, K. Akashi, D.G. Tenen, C/EBPbeta is required for 'emergency' granulopoiesis, Nat. Immunol. 7 (7) (2006) 732–739.
[26] M.S. Lionakis, J.K. Lim, C.C.R. Lee, P.M. Murphy, Organ-specific innate immune responses in a mouse model of invasive candidiasis, J. Innate Immun. 3 (2) (2011) 180–199.
[27] Y. Fu, Q.T. Phan, G.P.S. Luo, N.V. Solis, Y.P. Liu, B.P. Cormack, J.E. Edwards, A. S. Ibrahim, S.G. Filler, Investigation of the function of *Candida albicans* Als3 by heterologous expression in *Candida glabrata*, Infect. Immun. 81 (7) (2013) 2528–2535.
[28] M. Shi, S.S. Li, C. Zheng, G.J. Jones, K.S. Kim, H. Zhou, P. Kubes, C.H. Mody, Real-time imaging of trapping and urease-dependent transmigration of Cryptococcus neoformans in mouse brain, J. Clin. Investig. 120 (5) (1683-1693).
[29] F.H. Santiago-Tirado, M.D. Onken, J.A. Cooper, R.S. Klein, T.L. Doering, Trojan horse transit contributes to blood-brain barrier crossing of a eukaryotic pathogen, mBio 8 (1) (2017).
[30] J.F. Gibson, A. Bojarczuk, R.J. Evans, A.A. Kamuyango, R. Hotham, A.K. Lagendijk, B.M. Hogan, P.W. Ingham, S.A. Renshaw, S.A. Johnston, Blood vessel occlusion by Cryptococcus neoformans is a mechanism for haemorrhagic dissemination of infection, PLoS Pathog. 18 (4) (2022), e1010389.
[31] D. Sun, M. Zhang, P. Sun, G. Liu, A.B. Strickland, Y. Chen, Y. Fu, M. Yosri, M. Shi, VCAM1/VLA4 interaction mediates Ly6Clow monocyte recruitment to the brain in a TNFR signaling dependent manner during fungal infection, PLoS Pathog. 16 (2) (2020), e1008361.
[32] K. Bisht, K.A. Okojie, K. Sharma, D.H. Lentferink, Y.-Y. Sun, H.-R. Chen, J. O. Uweru, S. Amancherla, Z. Calcuttawala, A.B. Campos-Salazar, B. Corliss, L. Jabbour, J. Benderoth, B. Friestad, W.A. Mills, B.E. Isakson, M.-È. Tremblay, C.-Y. Kuan, U.B. Eyo, Capillary-associated microglia regulate vascular structure and function through PANX1-P2RY12 coupling in mice, Nat. Commun. 12 (1) (2021) 5289.
[33] K. Haruwaka, A. Ikegami, Y. Tachibana, N. Ohno, H. Konishi, A. Hashimoto, M. Matsumoto, D. Kato, R. Ono, H. Kiyama, A.J. Moorhouse, J. Nabekura, H. Wake, Dual microglia effects on blood brain barrier permeability induced by systemic inflammation, Nat. Commun. 10 (1) (2019) 5816.
[34] M.V. Russo, L.L. Latour, D.B. McGavern, Distinct myeloid cell subsets promote meningeal remodeling and vascular repair after mild traumatic brain injury, Nat. Immunol. 19 (5) (2018) 442–452.
[35] D.H.M.L.P. Navarathna, J. Munasinghe, M.J. Lizak, D. Nayak, D.B. McGavern, D. D. Roberts, Magnetic resonance imaging confirms loss of blood- brain barrier integrity in a mouse model of disseminated candidiasis, NMR Biomed. 26 (9) (2013) 1125–1134.

[36] C.-Y. Xu, H.-M. Zhu, J.-H. Wu, H. Wen, C.-J. Liu, Increased permeability of blood–brain barrier is mediated by serine protease during Cryptococcus meningitis, J. Int. Med. Res. 42 (1) (2014) 85–92.
[37] R. Patel, M.A. Hossain, N. German, A.J. Al-Ahmad, Gliotoxin penetrates and impairs the integrity of the human blood-brain barrier in vitro, Mycotoxin. Res. 34 (4) (2018) 257–268.
[38] E. Pasciuto, O.T. Burton, C.P. Roca, V. Lagou, W.D. Rajan, T. Theys, R. Mancuso, R. Y. Tito, L. Kouser, Z. Callaerts-Vegh, A.G. de la Fuente, T. Prezzemolo, L.G. Mascali, A. Brajic, C.E. Whyte, L. Yshii, A. Martinez-Muriana, M. Naughton, A. Young, A. Moudra, P. Lemaitre, S. Poovathingal, J. Raes, B. De Strooper, D.C. Fitzgerald, J. Dooley, A. Liston, Microglia require CD4 T cells to complete the fetal-to-adult transition, Cell 182 (3) (2020) 625–640, e24.
[39] E.A. Moseman, A.C. Blanchard, D. Nayak, D.B. McGavern, T cell engagement of cross-presenting microglia protects the brain from a nasal virus infection, Sci. Immunol. 5 (48) (2020).
[40] J.N. Jarvis, G. Meintjes, K. Rebe, G.N. Williams, T. Bicanic, A. Williams, C. Schutz, L.-G. Bekker, R. Wood, T.S. Harrison, Adjunctive interferon-γ immunotherapy for the treatment of HIV-associated cryptococcal meningitis: a randomized controlled trial, AIDS 26 (9) (2012) 1105–1113.
[41] D.B. Meya, Y.C. Manabe, D.R. Boulware, E.N. Janoff, The immunopathogenesis of cryptococcal immune reconstitution inflammatory syndrome: understanding a conundrum, Curr. Opin. Infect. Dis. 29 (1) (2016) 10–22.
[42] Y.M. Khaw, N. Aggarwal, W.E. Barclay, E. Kang, M. Inoue, M.L. Shinohara, Th1-Dependent cryptococcus-associated immune reconstitution inflammatory syndrome model with brain damage, Front. Immunol. 11 (2020).
[43] J. Xu, L.M. Neal, A. Ganguly, J.L. Kolbe, J.C. Hargarten, W. Elsegeiny, C. Hollingsworth, X. He, M. Ivey, R. Lopez, J. Zhao, B. Segal, P.R. Williamson, M. A. Olszewski, Chemokine receptor CXCR3 is required for lethal brain pathology but not pathogen clearance during cryptococcal meningoencephalitis, Sci. Adv. 6 (25) (2020) eaba2502.
[44] Z. Fitzpatrick, G. Frazer, A. Ferro, S. Clare, N. Bouladoux, J. Ferdinand, Z.K. Tuong, M.L. Negro-Demontel, N. Kumar, O. Suchanek, T. Tajsic, K. Harcourt, K. Scott, R. Bashford-Rogers, A. Helmy, D.S. Reich, Y. Belkaid, T.D. Lawley, D.B. McGavern, M.R. Clatworthy, Gut-educated IgA plasma cells defend the meningeal venous sinuses, Nature 587 (7834) (2020) 472–476.
[45] S.A. Liddelow, K.A. Guttenplan, L.E. Clarke, F.C. Bennett, C.J. Bohlen, L. Schirmer, M.L. Bennett, A.E. Münch, W.-S. Chung, T.C. Peterson, D.K. Wilton, A. Frouin, B. A. Napier, N. Panicker, M. Kumar, M.S. Buckwalter, D.H. Rowitch, V.L. Dawson, T. M. Dawson, B. Stevens, B.A. Barres, Neurotoxic reactive astrocytes are induced by activated microglia, Nature 541 (7638) (2017) 481–487.
[46] J.L. Zamanian, L. Xu, L.C. Foo, N. Nouri, L. Zhou, R.G. Giffard, B.A. Barres, Genomic analysis of reactive astrogliosis, J. Neurosci. 32 (18) (2012) 6391–6410.
[47] H. Sun, X. Wan, Y. Fan, P. Liu, Y. Song, N. Zhu, Z. Duan, Q. Wang, F. Chen, C. Zhou, Y. Zheng, P. Ding, F. Liu, L. Feng, K.S. Kim, L. Wang, Bacteria reduce flagellin synthesis to evade microglia-astrocyte-driven immunity in the brain, Cell Rep. 40 (1) (2022), 111033.
[48] S.K. Shrivastava, E. Dalko, D. Delcroix-Genete, F. Herbert, P.A. Cazenave, S. Pied, Uptake of parasite-derived vesicles by astrocytes and microglial phagocytosis of infected erythrocytes may drive neuroinflammation in cerebral malaria, Glia 65 (1) (2017) 75–92.
[49] K.M. Still, S.J. Batista, C.A. O'Brien, O.O. Oyesola, S.P. Früh, L.M. Webb, I. Smirnov, M.A. Kovacs, M.N. Cowan, N.W. Hayes, J.A. Thompson, E.D. Tait Wojno, T.H. Harris, Astrocytes promote a protective immune response to brain Toxoplasma gondii infection via IL-33-ST2 signaling, PLoS Pathog. 16 (10) (2020), e1009027.
[50] E.M. Eichenberger, J. Saullo, D. Brander, S.H. Wang, J.R. Perfect, J.A. Messina, A case of CNS aspergillosis in a patient with chronic lymphocytic leukemia on first-line ibrutinib therapy, Med. Mycol. Case Rep. 27 (2020) 17–21.
[51] T.H. Le, V. Kumar, K. Gondal, M. Barnes, H. Siddique, B. Buttar, A. Kaell, Isolated central nervous system Aspergillosis infection in a chronic lymphocytic leukemia patient on Ibrutinib: a case report, BMC Infect. Dis. 20 (1) (2020) 175.
[52] A. Bercusson, T. Colley, A. Shah, A. Warris, D. Armstrong-James, Ibrutinib blocks Btk-dependent NF-κB and NFAT responses in human macrophages during Aspergillus fumigatus phagocytosis, Blood 132 (18) (2018) 1985–1988.
[53] B.D. Snarr, R.A. Drummond, M.S. Lionakis, It's all in your head: antifungal immunity in the brain, Curr. Opin. Microbiol. 58 (2020) 41–46.
[54] J. Wheat, T. Myint, Y. Guo, P. Kemmer, C. Hage, C. Terry, M.M. Azar, J. Riddell, P. Ender, S. Chen, K. Shehab, K. Cleveland, E. Esguerra, J. Johnson, P. Wright, V. Douglas, P. Vergidis, W. Ooi, J. Baddley, D. Bamberger, R. Khairy, H.R. Vikram, E. Jenny-Avital, G. Sivasubramanian, K. Bowlware, B. Pahud, J. Sarria, T. Tsai, M. Assi, S. Mocherla, V. Prakash, D. Allen, C. Passaretti, S. Huprikar, A. Anderson, Central nervous system histoplasmosis: multicenter retrospective study on clinical features, diagnostic approach and outcome of treatment, Medicine 97 (13) (2018), e0245.
[55] J.R. Bariola, P. Perry, P.G. Pappas, L. Proia, W. Shealey, P.W. Wright, J. M. Sizemore, M. Robinson, R.W. Bradsher Jr, Blastomycosis of the central nervous system: a multicenter review of diagnosis and treatment in the modern era, Clin. Infect. Dis. 50 (6) (2010) 797–804.