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PEDIATRIC FUNGAL INFECTIONS (E ROILIDES, SECTION EDITOR)

Candidiasis of the Central Nervous System in Neonates and Children With Primary Immunodeficiencies

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Abstract

Purpose of review *Candida* infections of the central nervous system (CNS) are a life-threatening complication of invasive infections that most often affect vulnerable groups of patients, including neonates and children with primary immunodeficiency disorders (PID). Here, we review the currently known risk factors for CNS candidiasis, focusing predominantly on the PID caused by biallelic mutations in *CARD9*.

Recent findings How the CNS is protected itself against fungal invasion is poorly understood. CARD9 promotes neutrophil recruitment and function, and is the only molecule shown to be critical for protection against CNS candidiasis in humans thus far.

Summary Fundamental insights into the pathogenesis of CNS candidiasis gained from studying rare CARD9-deficient patients has significant implications for other patients at risk for this disease, such as CARD9-sufficient neonates. These findings will be important for the development of adjunctive immune-based therapies, which are urgently needed to tackle the global burden of invasive fungal diseases.

Keywords *Candida* · Candidiasis · CARD9 · Neutrophils · Brain · Neonates

Introduction

Neonates, especially those of low birth weight and/or preterm birth, are at greater risk for developing serious infections due to their under-developed immune systems. Fungal infections are particularly dangerous, since the suboptimal available diagnostic tools can lead to delays in initiation of antifungal therapy. As a result, invasive fungal infections of neonates are associated with unacceptably high mortality and morbidity rates [1, 2]. The most important invasive fungal infections affecting neonates are caused by *Candida* species, which are responsible for the largest number of infections [1]. *Candida albicans* is the most common infecting species, although recent years have seen a rise in the number of cases involving non-*albicans* species, including *C. parapsilosis* and *C. glabrata* [2].

Involvement of the central nervous system (CNS) is a major complication of invasive candidiasis in neonates [2]. Diagnosis of CNS candidiasis is fraught with difficulty, since signs and symptoms of CNS candidiasis are similar to other infections of the CNS (e.g. those caused by gram-positive or gram-negative bacteria) and analysis of cerebrospinal fluid (CSF) can often be misleading [3]. Early diagnosis and prompt antifungal therapy significantly improves the chances for survival; however, this currently requires a high degree of clinical suspicion [4]. Therefore, it is important that we aim to improve our understanding of the risk factors that predispose to CNS candidiasis, as well as identify the immune mechanisms that mediate protection from invasion in this sanctuary anatomical site.

In this mini-review, we discuss the incidence, risk factors, and pathogenesis of CNS candidiasis, focusing predominantly on the primary immunodeficiency disorder (PID) caused by deficiency in the C-type lectin receptor (CLR) adaptor molecule CARD9 (caspase recruitment domain-containing protein 9). In addition, we examine how recent insights from studying a rare PID, such as human CARD9 deficiency may help to develop a better understanding of the pathogenesis and protective therapies for a more common clinical entity, that seen in neonates who are at risk for developing CNS candidiasis.

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CNS Candidiasis: Incidence and Risk Factors

The incidence of invasive *Candida* infections in neonates ranges from 0.5 to 20% in the USA, with highest rates in low birth weight preterm births. CNS involvement has been reported in up to 60% of affected cases [1, 2, 4]. CNS candidiasis is associated with mortality rates of 35–40% [1, 2], in addition to an increased risk of neurodevelopmental disorders in survivors [1].

Few independent clinical risk factors for the development of CNS candidiasis in otherwise healthy neonates have been identified. Retrospective studies have shown that necrotizing enterocolitis is an important determinant of subsequent development of invasive fungal infection, accounted for permissive translocation of the commensal *Candida* population from the gastrointestinal tract into the bloodstream [1, 3]. The use of broad-spectrum antibiotics, central venous catheters, and respiratory *Candida* colonization are common clinical risk factors among infants who later develop CNS candidiasis [2]; however, none of these factors alone have been proven to significantly increase risk over invasion of non-CNS tissue.

Several single-nucleotide polymorphisms (SNPs) in immune-related genes have been found to significantly enhance risk of developing invasive *Candida* infections in recent years. We recently demonstrated that homozygosity for the dysfunctional *CX3CR1-M280* allele resulted in abrogated monocyte signaling and survival [5], and was an independent risk factor for developing systemic *Candida* infections [6]. In addition, the dysfunctional *CXCR1-T276* allele resulted in impaired neutrophil degranulation and fungal killing and was an independent risk factor for worse outcome after systemic *Candida* infection [7]. Analysis of large cohorts of patients and thousands of SNPs identified mutations in genes *CD58* and *TAGAP* and the LCE4A-C1orf68 locus as further independent risk factors for candidemia and invasive candidiasis [8]. Studies measuring levels of mannose-binding lectin (MBL) in the blood of patients with invasive candidiasis indicate that this molecule may also be involved in protection against these infections [9], and SNPs in MBL have been associated with the development of site-specific *Candida* infections in the vaginal mucosa and peritoneum [10, 11]. However, all of these SNPs do not specifically result in CNS-targeted disease. In contrast, the PID caused by CARD9 deficiency is the most concrete risk factor predisposing to the development of CNS-targeted candidiasis that has been identified in recent years.

CARD9 is a myeloid-expressed signaling adaptor protein involved in mediating the cellular responses of several members of the CLR superfamily of pattern-recognition receptors (PRRs), including Dectin-1, Dectin-2, Dectin-3, Mincle, and others [12]. CARD9-dependent functions include the phagocytosis and/or killing of fungi by neutrophils and monocytes, activation of the inflammasome, and production of pro-inflammatory cytokines and chemokines in response to fungal-specific stimulation [12].

Abrogation of CARD9 function results in a profound susceptibility to fungal infections by certain fungal microorganisms, including *C. albicans* and the ubiquitous mold *Aspergillus fumigatus*, in both mice and humans [13]. Human CARD9 deficiency is a rare autosomal recessive PID, caused by biallelic missense and/or nonsense mutations in *CARD9* [13]. CARD9-deficient patients experience debilitating, life-threatening fungal infections that most often develop in childhood, although adult-onset disease can also occur [13]. However, these patients appear able to generate effective immunity to other pathogens, since no increased susceptibility to bacterial, parasitic, or viral infections has been reported to date in these patients. This is in line with the observation that CARD9-deficient peripheral mononuclear cells respond normally when stimulated ex vivo with non-fungal agonists (e.g. LPS), and only exhibit a defect when activated with fungal cell wall components, such as curdlan or zymosan, or with heat-killed fungal organisms [14]. This specific susceptibility to fungal infections observed with CARD9 deficiency is unique, since other PIDs that predispose to systemic fungal infections (such as Job's syndrome due to STAT3 deficiency, *STAT1* gain-of-function mutations, chronic granulomatous disease due to mutations in the five subunits of the NADPH oxidase, or GATA2 haploinsufficiency) also predispose to other non-fungal infectious diseases. Even within the CLR signaling pathway, deficiencies in the CARD9-signaling partners MALT1 and BCL10 are associated with life-threatening viral and bacterial infections, in addition to mucosal candidiasis, although, of note, MALT1- and BCL10-deficient patients have not been thus far been reported to develop CNS candidiasis [15].

Interestingly, the susceptibility to fungal diseases observed with CARD9-deficiency is relatively narrow, being limited to specific fungal species which typically only affect the CNS, the oral mucosa and the skin/subcutaneous tissue [13]. Several cases of CNS candidiasis associating with CARD9 deficiency have now been described [16–20, 21••], with several patients experiencing the first symptoms in early childhood [13]. Many CARD9-deficient patients succumb to their infection due to overwhelming meningoencephalitis and/or obstructive hydrocephalus, highlighting the non-redundant critical role for CARD9 in protecting the CNS against *Candida*. In addition to CNS candidiasis, CARD9-deficiency has also been shown to predispose to CNS diseases caused by other fungal species, including *A. fumigatus* [22••] and some dematiaceous fungi and phaeohyphomycetes [23, 24], although these infections occur less commonly than *Candida* species. These non-*Candida* species can rarely cause CNS infections in CARD9-sufficient neonates, with the addition of *Mucorales* and the dimorphic fungi *Histoplasma capsulatum*, *Blastomyces dermatitidis*, and *Coccidioides posadasii* [25, 26]. As indicated earlier for CNS candidiasis, all of these fungal CNS

infections are challenging to diagnose in neonates and young children, and often have a poor clinical outcome.

Protection Against CNS Candidiasis: the Critical Role of Neutrophils

Neutrophils are the most important effector cell in the defense against invasive *Candida* infections, since depletion of these cells (neutropenia) profoundly increases the risk of developing invasive candidiasis in mice and patients [12]. In the CNS, neutrophils are recruited rapidly upon *C. albicans* infection and are essential for the clearance of fungal cells from this tissue [21••, 27]. We have previously shown that neutrophil recruitment to the fungal-infected CNS depends on CARD9 in both mice and humans, whereas recruitment to the bacterial-infected CNS or the fungal-infected kidney does not require CARD9 [21••]. Interestingly, this trafficking defect is extrinsic to the neutrophils themselves, since human and mouse *CARD9*$^{-/-}$ neutrophils are able to mount normal chemotaxis responses towards a variety of chemotactic stimuli ex vivo [21••, 22••]. Instead, production of neutrophil-targeted chemoattractants by CNS-resident cells and recruited myeloid cells is defective in the absence of CARD9 (Fig. 1). This data helps to explain, at least in part, why human CARD9-deficiency presents with CNS candidiasis, since these patients develop a CNS-neutropenia that severely limits their ability to fight fungal infection in this tissue. Moreover, the very small numbers of neutrophils that are recruited into the *Candida*-infected CNS in *CARD9*$^{-/-}$ patients have impaired effector function as shown by defective killing against unopsonized *Candida* yeast cells [16, 21••].

Several reports of CARD9-deficient patients who failed to recruit neutrophils to the *Candida*-infected CNS [13, 28] and *Aspergillus*-infected abdominal tissues [22••] have now been reported in the literature, further underscoring the importance of CARD9-dependent neutrophil recruitment for the protection against invasive fungal diseases. A molecular understanding of how CARD9 mediates this important protective function will be paramount to the development of adjunctive immune-based therapies that may protect at-risk groups, such as neonates, from CNS candidiasis. We have previously shown that production of CXCR2 ligands, CXCL1, and CXCL2, is critically dependent on CARD9, and that glial cells (microglia, astrocytes, oligodendrocytes) in the brain and recruited myeloid cells (neutrophils, monocytes) are important sources of these neutrophil-attracting chemokines during fungal infection [21••]. Ongoing work using conditional Card9-knockout mice will help shed light on the relative contribution of these cell compartments in chemokine production and neutrophil recruitment to the infected CNS. Similarly, CARD9-dependent production of CXCL1 from lung epithelial cells has been shown to protect against pulmonary *A. fumigatus* infections via the recruitment of neutrophils [29•]. Other molecules that promote neutrophil recruitment in response to fungal infection include the CARD9-coupled receptor Dectin-1, which is required for neutrophil accumulation in the peritoneum following fungal challenge [30], and the chemokine receptor CCR1 for neutrophil recruitment into the *C. albicans*-infected kidney [31]. Whether these and other PRRs and chemoattractant molecules also contribute towards CARD9-dependent neutrophil recruitment during CNS candidiasis is not known. Yet, by dissecting these mechanisms of susceptibility in human CARD9 deficiency, we can begin to understand what is required of the immune system to protect

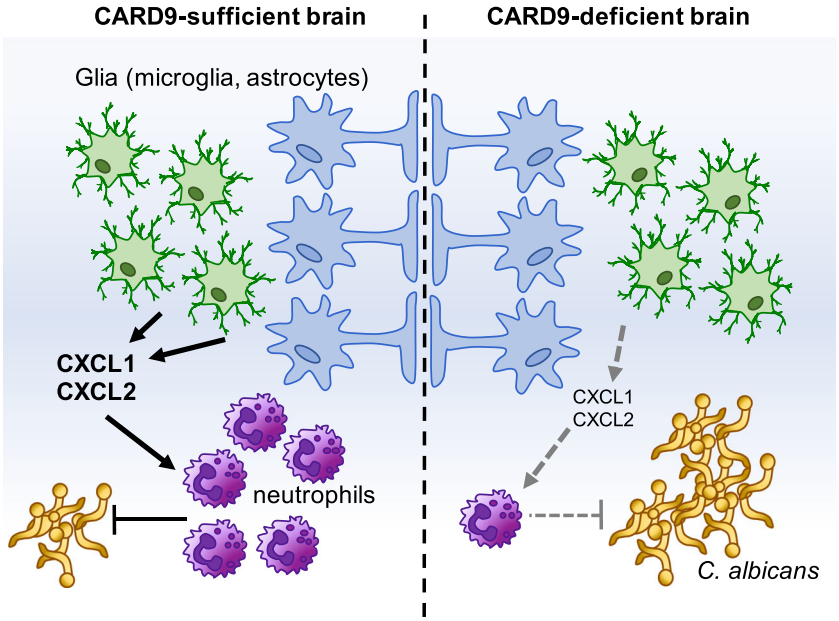

Fig. 1 CARD9 is a central regulator of neutrophil-dependent antifungal immunity in the CNS. In the CARD9-sufficient brain (left), resident glial cells produce CXC chemokines upon *C. albicans* infection, which drives neutrophil recruitment. Recruited neutrophils also produce CXC chemokines in the *C. albicans*-infected brain, thus amplifying neutrophil recruitment, which is necessary to control fungal growth in the CNS. In contrast, CARD9-deficient glial cells (right) have severe defects in the production of neutrophil-targeted chemokines, resulting in poor neutrophil recruitment and uncontrolled fungal growth

against CNS candidiasis which in turn can help those more commonly affected by this disease, such as CARD9-sufficient neonates.

The neonatal immune system is distinct to that of adults, defined by a severely under-developed adaptive immune system (due to lack of antigen exposure in utero) and a low-functioning innate immune system. Neonatal T cells are strongly skewed towards the Th2 phenotype, caused by a hyper-methylation status of the IFNγ gene and low production of Th1-polarizing cytokines and interferons from neonatal dendritic cells (DCs) [32]. These conditions favor Th2 immunity, which helps to prevent reactivity to antigens deriving from the mother [32]; however, this conversely enhances susceptibility to a variety of infections caused by gram-negative bacteria and fungi, which require Th1 and Th17 responses for effective immunity [12].

Of particular relevance to CNS candidiasis, neonates exhibit profound defects in neutrophil function which may contribute towards the development of this fungal disease. Human neonatal neutrophils have reduced chemotactic ability in ex vivo assays [33•], and animal models have revealed that neutrophils do not efficiently migrate into the neonatal brain following injury of both infectious and non-infectious origin [34•]. Similar to CARD9-deficient patients, poor neutrophil accumulation in the CSF has been reported in a number of cases of CARD9-sufficient neonatal CNS candidiasis [2, 4], whereas recruitment of neutrophils in neonates infected with bacterial pathogens appears intact [35]. Moreover, neonatal neutrophils express significantly reduced levels of genes involved in the IL-1 signaling pathway, production of iNOS, and activation of the inflammasome compared to older children [33•]. All of these pathways have been shown to be critical for the protection against fungal infections [27], and thus these immunologic disturbances could potentially further negatively impact the ability of these cells to fight *Candida* infection.

In addition to neutrophils, resident glial cells, such as microglia, also play critical roles in containing *C. albicans* infection within the CNS [21••]. Neonatal microglia rapidly mature upon seeding the developing brain and are important for various developmental processes including synaptogenesis and myelination [36]. Whether neonatal microglia are functionally impaired against fungi relative to adult microglia is not understood, however neonatal microglia have been shown to respond to inflammatory stimuli with greater magnitude than that of adult microglia [37], and this hyper-inflammatory phenotype has severe consequences for long-term functioning of the brain [38]. Whether similar pathways operate during neonatal CNS candidiasis and contribute towards the observed disabilities in survivors is not known. Future studies in wild-type neonatal versus adult mice infected with *Candida* should help shed light in age-dependent impairments in CNS resident glial functions, and in the recruitment and effector function of myeloid cells during CNS invasion.

Conclusions

The current treatment options available for CNS candidiasis, and invasive fungal infections more generally, involves administration of antifungal drugs, such as amphotericin B, fluconazole, and the newer triazoles or the echinocandins. However, some of these antifungal drugs, such as the echinocandins, have poor penetration into the CNS [3, 39, 40], and thus these treatments work most effectively in candidiasis when given before the infection reaches the CNS. This depends on early diagnosis, which is often not possible based on our current array of diagnostic tools that are not always effective in capturing early disease. Therefore, there is an urgent need for adjunctive immune-based therapies to treat those with invasive fungal diseases, including CNS candidiasis. Indeed, treatment of CARD9-deficient French-Canadians with recombinant GM-CSF was shown to result in clinical remission in these patients, by correcting the GM-CSF production defects observed in myeloid cells harboring the p.Y91H *CARD9* mutation [17, 41], while G-CSF therapy was shown to correct cytokine (IL-17) production defects in a CARD9-deficient patient with the p.Q295X mutation [42]. Therefore, adjunctive immune-based therapies such as these hold promise for treating these dangerous infections, and these studies demonstrate how studying rare PIDs can provide key insights into how the CNS is protected from fungal diseases. Such insights may lead to breakthrough discoveries that will significantly benefit susceptible groups of patients from dangerous fungal diseases, which are currently a significant burden in modern day health care.

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Compliance with Ethical Standards

Conflict of Interest No conflict to writing this review exists.

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