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Microglia and Neutrophils to the Rescue

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Abstract

Invasive candidiasis is a medical condition that is challenging to treat. A recent study by Lionakis and colleagues (*Nat Immunol*, 2019) reveals new insights into the role of microglia and neutrophils in limiting fungal dissemination to the mouse brain. Gaining knowledge into tissue-specific protection mechanisms may ensure improved prognosis and disease management against fungal infections.

Keywords

neutrophils; fungal infection; microglia; innate immunity

More than 3 million people acquire life-threatening invasive fungal infections annually (1). Despite treatments, these infections have 30–60% mortality rate (2). Their management is further complicated by the rise of drug resistance *Candida spp.* isolates. These facts are concerning and reveal that invasive candidiasis is a difficult-to-treat medical condition for which a better understanding of its pathogenesis at the host-pathogen interface is essential to improve patient outcomes.

Human deficiency in C-type lectin receptor (CLR)–spleen tyrosine kinase (Syk) adaptor CARD9 manifests with relapsing *C. albicans* meningoencephalitis. A variety of mutations contribute to the development of human CARD9 deficiency. One example is that of a patient carrying a T217C variant in the CARD domain that replaces an evolutionary conserved tyrosine residue to histidine. Candidiasis induces Th17 cells, and in this patient, despite comparable-to-normal Th17 cell numbers, the individual developed recurrent cerebrospinal candidiasis; this suggested that defects in non-Th17 pathways were present, affecting protective anti-fungal responses in the central nervous system (CNS) (3). GM-CSF is known to control monocyte/microglia proliferation and/or functionality and also govern neutrophil longevity. Thus, the patient was treated with granulocyte monocyte colony stimulating factor (GM-CSF) administration-- a cytokine whose expression depends on CARD9 (3,4). While the cellular and molecular mechanisms of the therapeutic effect of GM-CSF-based therapy

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were not investigated in that patient, the study implied that microglia had a potential role in protecting the CNS during CARD9 deficiency (3). Now, recent work by Drummond *et al.* sheds light on the role of microglia CARD9 in mediating neutrophil recruitment to the CNS during candidiasis (5).

In mouse models of intravenous *C. albicans* infection, Drummond and co-workers described a complex pathway of protective responses triggered by a *C. albicans*-secreted toxin, Candidalysin, which acted on microglia (but not on monocytes or neutrophils), eliciting the production of cytokines that recruited neutrophils to the infected brain, thus limiting pathogen spreading (5). Microglia cytokines interleukin IL-1 β and to a lesser extent, IL-1 α facilitated neutrophil recruitment by prompting the expression of chemokine (C-X-C motif) ligand 1 (CXCL1) by astrocytes, which in turn, attracted CXCR2-carrying neutrophils to the brain. Moreover, CARD9 deficient mice (*Card9*^{-/-}) showed profound defects in their ability to recruit neutrophils to the infected brain, resulting in a significantly elevated fungal presence relative to wildtype (WT) mice. Consistent with this result, CARD9-deficient microglia failed to respond to Candidalysin, with impaired secretion of IL-1 β . This suggested that resident microglia harbored key regulatory functions to guard brain tissues against invasive candidiasis.

The findings prompted many exciting follow-up questions. Because CARD9 relays signals downstream from CLRs, the authors investigated whether there might be any brain-specific mechanisms for fungal recognition. A defect in pattern recognition mechanisms upstream of CARD9 --specific to the CNS -- might explain the fungal CNS-tropism. To this end, the functional consequences of genetically-ablating Dectin-1, Dectin-2, Dectin-3, and Mincle in mice were analyzed. None of these deficiencies exhibited as strong a phenotype as CARD9 deficiency; this suggested that either alternative pathway(s) were involved in fungal recognition in the brain, or, that the cumulative absence of CLRs was required to reach the levels of susceptibility observed in CARD9-deficient mice. Of note, a recent candidiasis patient cohort study revealed that three single nucleotide polymorphisms (SNPs) in Toll-like receptor-1 (TLR1), but not in TLR2, TLR4, TLR6, TLR9, MyD88, or Toll/Interleukin-1 receptor domain-containing adapter protein (TIRAP) were associated with a heightened risk of invasive candidiasis relative to healthy individuals; this suggested that TLR1 signaling might predominantly contribute to optimal immunity against *C. albicans* in humans (6). However, whether or not TLR1-driven pathways cause CNS-specific recognition of *C. albicans* remains to be clarified.

Furthermore, in the recent study by the Lionakis group (5), when CARD9 was specifically deleted in microglia (*Card9*^{fl/fl}*Cx3cr1*^{CreER}^{-/-}), the infected mice showed only 2-times the level of reduction in neutrophil recruitment to the brain than *Card9*^{-/-} mice -- a defect mildly phenocopying that of global CARD9 deficiency (*Card9*^{-/-}), where reduced neutrophil recruitment to the CNS was significantly more dramatic than in WT mice. Consequently, although these data supported the significant contribution of microglia in protecting the CNS against candidiasis via a CARD9-dependent pathway, they also revealed that additional yet-to-be-discovered mechanisms are likely implicated. Indeed, given that the meninges harbor resident granulocytic populations at steady state (7), it is possible that CARD9 deficiency in those cell types further regulates neutrophil recruitment. Consistent

with this hypothesis, resident mast cells in the meninges have been shown to quickly recruit an early wave of neutrophils to the CNS in experimental autoimmune encephalomyelitis (mouse model for multiple sclerosis) (8). Thus, it is likely that mast cells -- which carry receptors that recognize *C. albicans*-derived factors -- may participate in recruiting neutrophils to the infected brain. Indeed, the impact of these resident immune cells on limiting fungal dispersal to brain tissues should be further evaluated.

Neutrophils are indispensable in host defense against candidiasis (9). They release reactive oxygen species to control yeast to hyphae transitions, and extrude neutrophil extracellular traps to limit fungal growth. Human mutations in CARD9 also result in a selective defect in fungal killing by neutrophils; hence, infected *Card9*^{-/-} deficient neutrophils can serve as a reservoir for initial *C. albicans* spread. It will be interesting to evaluate how the resident population of neutrophils in the meninges responds to fungi and orchestrates downstream events. Indeed, neutrophils can utilize a variety of mechanisms to secrete IL-1 β and hence, can further amplify neutrophil trafficking to infected sites. Moreover, the timely recruitment of neutrophils into the spleen and liver enables control over fungal growth. However, slow recruitment into infected kidneys correlates with significant pathology (10). Along these lines, Lionakis and colleagues have demonstrated organ-specific cellular responses (5). It is likely that similar mechanisms of CNS protection against other fungal pathogens such as *Cryptococcus neoformans* and *Aspergillus fumigatus*, exist, but these remain to be investigated.

In conclusion, *Candida* is a commensal organism which normally colonizes 50% of humans; however, under conditions of immunosuppression, *Candida spp.* can transform into life-threatening opportunistic pathogens causing invasive disease. Dissecting tissue-specific mechanisms of protection and recognition of fungi might allow the design of novel risk analyses, prognosis, as well as putative vaccination and therapeutic strategies in patients suffering from fungal infections that disseminate to the brain.

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