Regulation of C-Type Lectin Receptor-Mediated Antifungal Immunity

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Of all the pathogen recognition receptor families, C-type lectin receptor (CLR)-induced intracellular signal cascades are indispensable for the initiation and regulation of antifungal immunity. Ongoing experiments over the last decade have elicited diverse CLR functions and novel regulatory mechanisms of CLR-mediated-signaling pathways. In this review, we highlight novel insights in antifungal innate and adaptive-protective immunity mediated by CLRs and discuss the potential therapeutic strategies against fungal infection based on targeting the mediators in the host immune system.

Keywords: C-type lectin receptor, fungal infection, signaling pathways, posttranslational modifications, immunity

INTRODUCTION

Fungi are ubiquitously present in the mucosal and epidermal surfaces in healthy individuals and often cause infections in immune-compromised patients. These include HIV-positive patients, recipients of organ transplants, and cancer patients treated with chemotherapy. In healthy individuals, fungal infections can also develop including vulvovaginal candidiasis, tinea pedis, fungal keratitis, and chromoblastomycosis (1–6). Invasive fungal infections, particularly with Candida albicans (C. albicans), demonstrate high mortality rates and kill more than 1.5 million people worldwide annually (7). Moreover, other identified pathogenic fungi such as Aspergillus fumigatus (A. fumigatus), C. auris, and Cryptococcus gattii (C. gattii) also pose a great threat to public health (1, 8, 9). Toxicity and resistance to the limited number of antifungal agents that are currently available contribute to high morbidity and mortality associated with fungal sepsis. Therefore, there is an urgent need to better understand the immune response during fungal infection and develop new immuno-therapeutic approaches.

C-type lectin receptors (CLRs), including transmembrane and soluble forms, are characterized by containing at least one C-type lectin-like domain (CTLD). They have been shown to recognize both endogenous and exogenous ligands (10). As the most important pattern recognition receptor (PRR) family for the detection of fungi, CLRs are recognized to play a critical role in tailoring immune responses against fungal exposure (11–14). In this review, we will focus on the roles and mechanisms of membrane-bound CLR-mediated-signaling pathways in host defense against fungal infections, with an emphasis on C. albicans. C. albicans is the most common fungal species isolated from biofilms, formed either on implanted devices or on human tissues, which become pathogenic in immune-compromised patients. We will also discuss the role of posttranslational modifications (PTMs) of CLR-signaling pathway components in anti-fungal immunity. In addition, we will also summarize the recent progress on the potential host-derived immune therapies for disseminated candidiasis.
Fungal Recognition

The pathogen-associated molecular patterns (PAMPs) on the fungal cell wall are crucial for the initiation of innate immune responses against fungal pathogens. The fungal cell wall is predominantly composed of carbohydrate polymers interspersed with glycoproteins (15–17). The three major components, found in almost all fungi, are β-glucans, which are anchored in the inner core of the cell wall, chitin, which is a robust β-1,4-linked homopolymer of N-acetylglucosamine (GlcNac) located in the inner cell wall, and mannans, which are localized in the outer layer of the fungal yeast cell wall. The central core of the cell wall is branched β-1,3/1,6-glucans that are linked to chitin via β-1,4 linkages (15, 18). Mannans are chains of up to several hundred mannoses that are added to fungal proteins via N- or O-linkages. Mannoproteins can covalently attach to glucans or chitin via either their sugar residues or glycosylphosphatidylinositol (17). In addition, O-linked glycoproteins containing mannobiose-rich structures from Malassezia function as distinct ligands to induce immune responses (19). Another crucial component on the fungal cell wall is melanin, which is involved in fungal virulence, resistance to antifungal drugs, and protection against insults from the environment (20, 21).

The recognition of PAMPs expressed in pathogens involves four families of PRRs, including Toll-like receptors (TLRs), NOD-like receptors (NLRs), retinoic acid-inducible gene I (RIG-I)-like receptors (RLRs), and CLRs, each of which shows notable differences regarding pathogen recognitions, signal transduction, and intracellular downstream pathways (14, 22). Among these PRRs, CLRs have been shown to be essential for fungal recognition, either alone or in conjunction with TLRs (23–25). The family of CLRs comprises a subset of CTLD-containing proteins, including some Ca2+-dependent and Ca2+-independent carbohydrate-binding membrane-bound receptors. They are preferentially expressed by myeloid cells (26, 27). Ca2+-dependent carbohydrate binding is the most common CTLD function in vertebrates. Under these circumstances, the CTLD is therefore named as a carbohydrate recognition domain (CRD) (27). CLRs can recognize an array of molecules such as carbohydrates, proteins, and lipids.

Several PRRs have been reported to recognize β-glucans, including Dectin-1, complement receptor 3 (CR3), and three members of the scavenger receptor family, CD5, CD36, and SCARF1 (28–33). Recent studies have revealed that Dectin-2, Dectin-3, macrophage mannose receptor (MR), macrophage-inducible C-type lectin (Mincle), and dendritic cell (DC)-specific ICAM3-grabbing non-integrin (DC-SIGN) can recognize mannans and mannoproteins (12, 13, 34–37). It has been shown that Dectin-1 specifically recognizes β-1,3-glucans (11, 38), whereas Dectin-2 and Dectin-3 specifically recognize α-mannans (12, 13). The receptor(s) that recognizes chitin is still unknown, although NOD2, MR, and TLR9 were proposed to recognize chitin (39).

CLRs in Antifungal Immune Response

The major CLRs that are recognized to be involved in antifungal immune responses are Dectin-1, Dectin-2, Dectin-3, MR, Mincle, and DC-SIGN. Dectin-1, Dectin-2, Dectin-3, Mincle, and DC-SIGN share a similar molecular structure consisting of a CRD, a stalk region, a transmembrane domain, and a cytoplasmic domain (40–42). By contrast, MR is composed of an amino terminal cysteine-rich domain, a single fibronectin type II domain, eight CRDs, a transmembrane domain, and a cytoplasmic tail (43, 44) (Figure 1). These CLRs can be divided into two main groups based on their intracellular-signaling motifs: CLRs with immunoreceptor tyrosine-based activation motifs (ITAMs) or ITAM-like (also named hem-ITAM) domains and CLRs containing non-immunoreceptor tyrosine-based motifs such as MR and DC-SIGN (27, 45). The activation of these receptors can transduce intracellular-signaling pathways directly through integral ITAM-like motif(s) within the cytoplasmic tails (such as Dectin-1), or indirectly through association with ITAM-containing FcR-γ chains, including Dectin-2, Dectin-3, and Mincle (25, 27, 46). Upon ligand binding, the activation of receptors induces the tyrosine phosphorylation of ITAM-like/ITAM motif(s) by Src family kinases, leading to the recruitment and activation of Syk kinase. This subsequently initiates downstream-signaling pathways. The activation of Syk signaling requires its interaction with two phosphorylated tyrosines within ITAM-like/ITAM motifs. However, unlike canonical ITAM motifs within FcR-γ adaptors which contain a repeat of YxxL/I, the ITAM-like motif located in the cytoplasmic tail of Dectin-1 has only a single YxxL/I; thus, the signaling from Syk may involve the receptor dimerization of Dectin-1 containing a single phosphotyrosine (25, 47) (Figure 1). Signals from the CLRs initiate and modulate not only innate immune responses but also the development of adaptive immunity, especially Th1 and Th17 responses, which are crucial for the control of fungal infections. The role of Dectin-1, Dectin-2, and Dectin-3 in antifungal immunity is of considerable interest and has been extensively studied during the last decade.

Signaling Pathways Mediated by Dectin-1

Dectin-1 (encoded by Clec7a), which is mostly expressed by myeloid phagocytes (macrophages, DCs, and neutrophils), recognizes β-1,3-glucans in a calcium-independent manner (40). The engagement of Dectin-1 by β-1,3-glucans induces the activation of Src protein tyrosine kinase (PTK). Src phosphorylates the single cytoplasmic ITAM-like domain of Dectin-1, which subsequently results in the recruitment and activation of Syk (25, 48, 49). The activated Syk then phosphorylates protein kinase Cδ (PKC-δ), which phosphorylates caspase activation and recruitment domain-containing protein 9 (CARD9) (50, 51). This facilitates complex formation with Bcl-10 and MALT1 (51, 52), thus eliciting NF-κB activation (24, 51) (Figure 1). In addition, Dectin-1-induced activation of extracellular signal-regulated protein kinase (ERK) is also mediated through CARD9, which links Ras-GRF1 to H-Ras (53). In addition to the Syk-dependent pathways, signaling from Dectin-1 also involves Syk-independent pathways mediated by Raf-1, resulting in noncanonical NF-κB activation by collaboration with the Syk-dependent NF-κB-inducing kinase (NIK) pathway (24). The activation of NF-κB and ERK mediates the inflammatory responses against fungal infections and directs Th1/Th17 differentiation for antifungal immunity (24, 54–56).
Dectin-1 signaling induces numerous signaling events characterized by phagocytosis, respiratory burst, and the production of various inflammatory mediators, including cytokines, chemokines, and inflammatory lipids (40, 47, 57, 58). It is well established that the production of IL-1β, together with IL-6 and IL-23, is essential for antifungal immune responses partly through priming adaptive immunity to differentiate CD4+ T cells to TH1/TH17 cells (24, 56, 59). Moreover, the production of type I interferons (IFNs) can be induced after fungal recognition and require Dectin-1/Syk signaling and transcription factor IRF5 involvement (49). In mouse models, enhanced IFN-β secretion is critical for protection from fungal challenge (49, 60). Importantly, consistent with animal studies, type I IFNs exert a protective role against *C. albicans* infection in humans (61). Dectin-1 signaling also triggers the activation of nuclear factor of activated T cells (NF-AT) in infected mice (68). Dectin-1 signaling also results in the impairment of mucosal fungal defense, partly by inhibiting TH17-induced immune responses, which are responsible for the susceptibility to chronic mucocutaneous candidiasis (67). However, the underlying mechanism regarding how human CARD9 mutations affect TH17 immunity deserves further investigation.

Strikingly, CARD9 serves as the only currently known human gene in regulating the dissemination of *C. albicans* to the central nervous system (CNS). CARD9 deficiency in both mice and humans results in vulnerability to fungal infection in the CNS, owing to impaired neutrophil accumulation in the fungal-infected CNS, which correlates with the lack of CXC-chemokine induction (68). Consistent with these findings, decreased neutrophil...
recruitment to the lungs was reported in Card9−/− mice infected with A. fumigatus (69). CARD9 deficiency may also predispose to extrapulmonary A. fumigatus infection in humans as a result of impaired neutrophil recruitment (70). Nevertheless, the CARD9-dependent-protective role seems less necessary for pulmonary mold infections. Thus, future studies are required to decipher the role of CARD9 in other immune cells to explain its “organ-specific” and “species-specific” function in antifungal immunity. Moreover, the effects of genetic mutations in CARD9-coupled receptors (Dectin-1, Dectin-2, and Dectin-3) and CARD9-binding partners (MALT1 and Bcl-10) in human antifungal host defense require more in-depth studies.

Vav proteins, the key upstream regulators of CARD9, are critical in CLR/CARD9-induced-inflammatory responses similar to CARD9 (71). Indeed, humans with polymorphisms in DECTIN-1 and VA V3 show increased susceptibility to invasive C. albicans infection (71, 72). Interestingly, one recent study illustrated that neutrophilic myeloid-derived suppressor cells (MDSCs) are induced in vitro upon infection with various Candida species, which functionally inhibit T cell responses via Dectin-1/CARD9 signaling and subsequently suppress ROS generation, indicating that CARD9 seems to function as a negative modulator in fungal immune response (73). It is unknown whether this is true in vivo. The contribution of MDSCs to fungal infections requires further investigation.

### Signaling Pathways Mediated by Dectin-2, Dectin-3, and Mincle

Dectin-2 (encoded by Clec4n), Dectin-3 (MCL, encoded by Clec4d), and Mincle (encoded by Clec4e) belong to the Dectin-2 family of CLRs, whose encoding genes are grouped closely at the telomeric end of the NK-gene cluster. They all have a single extracellular CTLD, short cytoplasmic tails, and trigger intracellular signaling indirectly through association with the ITAM-containing FcR-γ chain (55, 74–76). Signaling from Dectin-2, Dectin-3, and Mincle is mediated via the Syk/PKCδ-dependent CARD9/Bcl-10/MALT1 pathway, resulting in the activation of the transcription factor NF-κB and the subsequent production of inflammatory cytokines and chemokines (13, 75, 77). Dectin-2 recognizes high-mannose structures and binds Candida α-mannans in a calcium-dependent manner (12, 78). It can also recognize O-linked mannobiose-rich glycoprotein from Malassezia, glycans containing mannose from house dust mite extracts (19, 79). Dectin-2 has been implicated in the defense against numerous pathogens, including C. albicans, C. neoformans, A. fumigatus, Saccharomyces cerevisiae, Paracoccidioides brasiliensis, Histoplasma capsulatum, Microsporum audouini, Trichophyton rubrum, Mycobacterium tuberculosis, and Schistosoma mansoni (75, 78, 80). Dectin-2 and Dectin-3 can form heterodimers to recognize the hyphal forms of C. albicans to induce pro-inflammatory cytokine production (13), although the involvement of Dectin-3 in C. neoformans infection is still controversial (81).

Mice deficient for Dectin-2 are highly susceptible to systemic candidiasis (12). Further study indicates that Dectin-2 and Dectin-3, two similar CLRs, form a constitutive heterodimeric PRR for sensing α-mannans on the surface of C. albicans and induce Syk-mediated activation of NF-κB to combat fungal invasion (13). Blocking either Dectin-2 or Dectin-3 with antibodies dramatically eliminates NF-κB-mediated-inflammatory responses upon C. albicans stimulation. The genetic deletion of Dectin-3, or mice receiving Dectin-3-blocking antibodies, showed high susceptibility to systemic candidiasis (13). Therefore, Dectin-2 coupled with Dectin-3 displays protective antifungal immunity in animal models. Recently, two studies also showed that Dectin-3 is constitutively expressed in myeloid cells and functions as an FcR-γ-coupled receptor for sensing trehalose-6,6′-dimycolate (TDM), a potent mycobacterial adjuvant (76, 82). In addition, Dectin-3 is also essential for inducing Mincle expression upon TDM stimulation (82). Dectin-3 has been shown to interact with Mincle via the stalk region of Dectin-3, thus enhancing the protein expression of Mincle (83).

Emerging evidence showed that the engagement of the TLR7/IL-17 pathway plays a critical role in host defense against mucosal fungal infection (84). Both Dectin-2 and Dectin-3 are of great importance for TLR7 and TLR8 cell differentiation in host defense against C. albicans or Blastomyces dermatitidis (12, 85). Furthermore, PI3K-δ, a proximal Syk-dependent-signaling intermediate downstream of Dectin-2, plays an important role in the generation of TLR2 and TLR7 immunities against infection with Dermatophagoides farina (D. farina) (86). In addition, a recent study showed that NF-κB subunit c-Rel-dependent cytokine induction relies on the Dectin-2/MALT1-signaling cascade to trigger TLR7-17-polarizing cytokines IL-1β and IL-23 secretion, thus possessing TLR7-17-protective immunity against pathogenic fungal invasion (52). The expression of IL-17RC on humans and murine neutrophils has been identified in a Dectin-2-dependent pathway (87). Dectin-2-induced autocrine IL-17 secretion has also been implicated with ROS generation and fungal killing (87).

Mincle has been shown to recognize mycobacteria, C. albicans, Malassezia, and Fonsecaea species (36, 88–90). It is the sensor for α-mannose, glycolipid trehalose-6, 6′-dimycolate (TDM), and the self-ribonucleoprotein SAP-130 (74, 88, 91). Mincle is expressed constitutively at low levels in myeloid cells, and its expression is dependent on Dectin-3 (76, 83, 92). The expression pattern of Mincle suggests that it may not be the major fungal recognition receptor. In support of this notion, although mice lacking Mincle display increased fungal burden in the kidneys, the survival rate of Mincle−/− mice is similar to wild-type mice upon systemic C. albicans infection (89). It has been shown that Mincle is not a phagocytic receptor but modestly potentiates pro-inflammatory cytokine production (89). Mincle has been shown to inhibit Dectin-1-induced TLR1 responses to F. monophora infection by inducing IRF1 degradation through the E3 ubiquitin ligase Mdm2, which impairs the polarization of TLR1 cells. Defective TLR1 responses contribute to the chronic infection of F. monophora which causes chromoblastomycosis, a chronic fungal skin infection (93). In addition, Mincle has been demonstrated to specifically recognize Malassezia species and play a crucial role in host defense against this fungus (36).
Other Fungal Recognition Receptors

MR (CD206, encoded by Mrc1): MR recognizes N-linked mannan of infectious Candida and mediates endocytosis and phagocytosis (94). Recent studies indicate that MR might promote the secretion of pro-inflammatory cytokines through the activating intracellular signal cascades. Although non-ITAM motifs are identified within the MR, a recent study reports that human MR becomes tyrosine phosphorylated upon M. tuberculosis (M. tb) infection, and this phosphorylation mediates a sequential association of Grb-2 and SHP-1 (95), suggesting that human MR itself can transduce downstream signaling. However, no known signaling motifs in murine MR have been identified, and no signaling has been induced directly from murine MR in response to fungal infections. Using human peripheral blood mononuclear cells, MR was found to be the main receptor pathway for the induction of T\textsubscript{H}17 cells by C. albicans in vitro (34). However, the importance of MR in fungal recognition is challenged by the fact that normal host defense is not altered during systemic candidiasis or Pneumocystis carinii infection in Mr\textsuperscript{-/-} mice (96). In support of this, MR is also not required for resistance to Coccidioides immitis infection (97). Therefore, MR may not be the major fungal recognition receptor in mice. It is possible that the human and murine MRs may behave differently. This notion is supported by a recent report that human but not mouse MR signaling induced by M. tb regulates macrophage recognition and vesicle trafficking (95).

DC-SIGN (encoded by Cld209a): DC-SIGN is a transmembrane receptor for pathogen binding and uptake, which is mainly expressed in a subset of macrophages and DCs (98, 99). It has been demonstrated that DC-SIGN can bind and internalize soluble ligands effectively, which facilitates antigen processing and presentation to T cells (100). DC-SIGN has a high affinity to detect varied carbohydrate-based ligands, including mannose structures and fucose-bearing glycans, to recognize diverse organisms including HIV-1, M. tb, Helicobacter pylori, or fungi such as C. albicans, A. fumigatus, and C. tropicalis (35, 37, 101–103). The polymorphisms of both Dectin-1 and DC-SIGN were reported to associate with invasive pulmonary Aspergillosis infection (104). It has been demonstrated that DC-SIGN can recognize Candida mannan and that N-linked mannosyl residues are essential for this interaction (37). In particular, the N-mannosylation is required for the binding, phagocytosis, and immune sensing of C. albicans by human DCs (37). Upon high-mannose recognition, the signalosome leads to Raf-1 activation and subsequent p65 acetylation, which facilitates gene-transcriptional expression, especially amplifying TLR-induced cytokine production such as IL-10, IL-6, and IL-12 (23). However, DC-SIGN in collaboration with MR seems to suppress Dectin-1-mediated T\textsubscript{H}17 responses, but potentiate T\textsubscript{H}1 responses in β-glucan- or M. tb-treated DCs (105). Moreover, DC-SIGN, which contains abundant galactomannan, is also found to play an important role in the recognition and binding of A. fumigatus conidia in human DCs (103). The ligation of DC-SIGN by the glycoprotein fimbriae of Porphyromonas gingivalis promotes the evasion of antibacterial autophagy and lysosome fusion, resulting in intracellular persistence in myeloid DCs, whereas TLR2 activation can overcome autophagy evasion and pathogen persistence in DCs (106). However, the importance of DC-SIGN in antifungal immunity has not been verified by a gene-targeting approach.

CD23 (encoded by Fcer2a): CD23 is the low-affinity receptor for IgE and is also a novel CLR which binds to α-mannans and β-glucans (107). A recent study illustrated that c-Jun N-terminal kinase 1 (JNK1) deficiency exerts a protective effect in systemic candidiasis. The expression of CD23 is negatively regulated through a Dectin-1-induced NF-AT pathway (107). Antifungal effector NOS2 is dramatically augmented through the recognition of α-mannans and β-glucans with CD23 in mice lacking JNK1. Likewise, the genetic deletion of CD23 abrogates the protection of Jnk\textsuperscript{1+} mice from disseminated candidiasis. JNK inhibitors boost antifungal innate immunity in vivo and in vitro (107). Taken together, JNK inhibition may also be a novel therapeutic strategy to combat disseminated candidiasis (Figure 2).

CR3 (Mac-1, α\textsubscript{M}G, or CD11b/CD18): CR3 is mainly expressed in leukocytes. CR3 consists of an I domain and a specific lectin domain, which bind to protein ligands such as iC3b, fibronectin and ICAM-1, and complement deposited on β-1,6-glycans (108). CR3 has been shown to cooperate with Dectin-1 for the detection of β-glucans and the regulation of innate immune responses during fungal pathogen exposure (109). Recent studies identified that CR3 and Dectin-1 collaboratively induce cytokine responses in macrophages in an Syk/JNK/AP-1 manner upon disseminated H. capsulatum infection (110), which further facilitates fungal-adaptive immune responses. Still, the underlying molecular mechanisms of crosstalk among other fungal PRRs will be of great interest for future investigations.

Collaboration between CLRs and TLRs

There is emerging evidence that signaling from CLRs in collaboration with other PRRs, especially TLRs, is indispensable for optimal antifungal immunity. It has been reported that the cooperative interaction between Dectin-1 and TLR2 or TLR4 synergistically facilitates the production of TNF, IL-23, and IL-10, but reduces IL-12 (59, 111). Dectin-1/TLR2 can amplify MR-mediated Th17 responses and IL-17 production upon C. albicans infection (34). In addition, DC-SIGN modulates the signaling from multiple TLRs on human DCs through activating Raf-1-dependent acetylation of NF-κB, which can promote the transcription of IL-10 and enhance antifungal-inflammatory response (23).

Recent studies have shown the importance of costimulation of Mincle and TLRs in protective antifungal response to F. pedrosoi, the most common fungus associated with chromoblastomycosis. Normally, F. pedrosoi is recognized by CLRs, but not TLRs, leading to the defective production of stimulatory cytokines and impaired fungal clearance. Intriguingly, the exogenous application of TLR7 ligand, imiquimod, restores the induction of inflammatory responses mediated via both Syk/CARD9- and MyD88-dependent-signaling pathways, as well as facilitates F. pedrosoi clearance in mice (90). In support of this finding, the topical administration of imiquimod to several patients with chromoblastomycosis also results in rapid infection resolution and greatly improved the lesions (112).
Emerging evidence shows that the engagement of inflammasomes plays a critical role in host defense against fungal infection, which can lead to the processing and activation of IL-1β and IL-18 (113). Both cytokines are implicated in mediating antifungal cellular responses, especially the promotion of adaptive Th1/Th17 responses.

NLRP3 has been proposed to be the main inflammasome involved in protective fungal immunity (114). Several CLRs and TLRs can induce the priming of inflammasomes and the activation of NF-κB via the recognition of fungal PAMPs, resulting in the expression of pro-IL-1β and pro-IL-18 (115–117). Both Dectin-1/Syk- and TLR2/MYD88-signaling pathways have been shown to induce NLRP3 priming in murine macrophages infected with C. albicans (114). In addition, the production of pro-IL-1β in response to A. fumigatus, M. canis, Malassezia spp., P. brasiliensis, and C. neoformans requires Dectin-1/Syk-dependent signaling (118–122). A more recent study indicates that Dectin-2 is the primary receptor for NLRP3 inflammasome activation in DCs in response to H. capsulatum (117). It is unknown whether Dectin-2 and other CLRs such as Dectin-3, Mincle, and MR are also involved in the activation of inflammasomes.

The canonical NLRP3 inflammasome can be triggered by ROS, K+ efflux, and lysosomal cathepsins release induced by various fungal species. Upon infection with C. albicans and A. fumigatus, it has been shown that the activation of the NLRP3 inflammasome requires transition from the yeast to the filamentous phase (123), which may be attributed to the differential exposure of β-glucans on the fungal surface and thus the differential recognition by Dectin-1 (124). Upon phagocytosis by host macrophages, C. albicans–secreted aspartic proteases Sap2 and Sap6 are thought to activate the caspase-1-dependent NLRP3 inflammasome by inducing ROS production and K+ efflux (126). Recent evidence has shown that NLRP3 coupling with AIM2 receptors is required to activate caspase-1- and caspase-8-dependent inflammasomes and induce protective antifungal responses in DCs challenged with A. fumigatus (127). Mice deficient in both NLRP3 and AIM2 are more susceptible to invasive Aspergillosis than mice lacking a single inflammasome receptor, suggesting the importance of cooperative activation and dual cytoplasmic surveillance of these two inflammasomes against A. fumigatus infection (127). Interestingly, mucosal Candida infection induces the activation of an NLRC4-dependent inflammasome, which can utilize caspase-1 to process IL-1β and IL-18 (128). The NLRC4
inflammation has not been confirmed by a gene-targeting approach. Furthermore, the role of caspase-8 in controlling the regulation of immune responses against fungi remains to be determined. Subsequently, PTMs regulate the activation of caspase-8 appears to be involved in the cleavage of pro-IL-1β and the production of its bioactive form to defend against fungi (115). A subsequent study reported that this noncanonical caspase-8 inflammamome can be activated and modulated by Tec, an intracellular non-receptor PTK, which acts as a novel signaling mediator between Dectin-1/Syk and PLC-γ2 in macrophages upon infection with Candida (116). The genetic ablation or the chemical inhibition of Tec results in a dramatic reduction of inflammatory responses and protects from fatal fungal sepsis (116). Interestingly, it has been shown that caspase-8, coordinating with caspase-1, plays a crucial role in promoting NLPR3 inflammamome-dependent maturation of IL-1β mediated by Dectin-1 and CR3 in DCs during β-glucan sensing and C. albicans infection (109). In addition, the same group also showed that there is crosstalk between CR3 and Dectin-1 during H. capsulatum yeast infection in macrophage TNF and IL-6 responses in an Syk/JNK/AP-1-dependent manner (110). However, it was reported that H. capsulatum α-(1,3)-glucan blocks innate immune recognition by Dectin-1 (129). The importance of Dectin-1 in H. capsulatum infection, in particular in vivo, remains to be determined. Furthermore, the role of caspase-8 in controlling antifungal immunity has not been confirmed by a gene-targeting approach.

CLR-MEDIATED PTMS IN ANTIFUNGAL IMMUNITY

Recent literature has shed additional light on novel molecules engaged in antifungal immunity and PTMs in CLR-signaling cascades, thus opening new avenues for innovative therapeutic approaches (107, 130–133). It has been increasingly recognized that PTMs serve as modulators to tailor fungal evasion by targeting innate sensors, adaptors, signaling components, and transcription factors. Subsequently, PTMs regulate the activation, survival, and stability of potent proteins by linking covalent bonds to functional groups (134). To date, several PTMs including phosphorylation and ubiquitination have been characterized in the regulation of immune responses against fungi.

Protein Kinases and Phosphatases in Antifungal Innate Immunity

Two major cytoplasmic kinase families in innate cells, including the Src family kinases and the Syk, are involved in intracellular-signaling cascades upon fungal pathogen exposure. Signaling involving the phosphorylation of tyrosine residues within the ITAM(s) by Src family kinases leads to the recruitment and activation of Syk, which then phosphorylates phospholipase Cγ2 (PLC-γ2). Activated PLC-γ2 initiates the hydrolysis of membrane-bound phosphatidylinositol-3,4,5-triphosphate (PIP3) to soluble inositol triphosphate (IP3) and diacylglycerol, both of which result in the influx of calcium and the activation of PKC-δ, the latter mediating the phosphorylation of CARD9 and the subsequent activation of the CARD9/Bcl-10/MALT1 complex. Downstream signaling through the Syk/PLCγ2 pathway from Dectin-1 and Dectin-2 involves the activation of NF-κB, ERK, and NF-AT (24, 53, 135, 136).

Numerous studies have shown that the balance between phosphorylation and dephosphorylation is of great importance in orchestrating fungal immune responses. In addition to Src kinases phosphorylating ITAM(s) within Dectin-1 and FcRγ, recent literature has shown that two members of Src family kinases, Fyn and Lyn, facilitate the cryptococcal-killing capacity in NK cells by mediating PI3K/ERK1/2-signaling activity, which further directs the traffic of perforin-containing granules to synapses for pathogen clearance (137). Whether other Src family kinases are involved in the regulation of fungal invasion remains to be determined.

The CARD9/Bcl-10/MALT1 complex in Dectin-1 signaling upon Candida infection has been known to activate the IKK complex, leading to the phosphorylation of IκB and the activation of all canonical NF-κB subunits including p50, p65, and c-Rel (24, 51, 138). Importantly, Syk activation in response to Dectin-1 stimulation is also able to activate noncanonical subunits of NF-κB (p52 and RelB) through NIK and IKKα, leading to the nuclear translocation of p52–RelB dimers (24). In addition, Dectin-1 signaling can induce Syk-independent phosphorylation and activation of Raf-1 via Ras. Activated Ras leads to the activation of Raf-1, which then phosphorylates NF-κB p65, and facilitates p65–RelB dimer formation that sequesters active RelB and potentiates Tnfα responses by inducing IL-12p40 and IL-1β (24). Interestingly, another group recently found that Dectin-1 stimulated with C. albicans triggers Syk-dependent phosphorylation of Ras-GRF1, which mediates the recruitment and activation of H-Ras through CARD9 alone, but not the CARD9/Bcl-10/MALT1 complex, leading to the phosphorylation and activation of ERK, but not NF-κB and subsequent pro-inflammatory responses (53). This suggests that upon Dectin-1 signaling, CARD9 is required for ERK activation but is dispensable for NF-κB activation.

CLR (Dectin-1, Dectin-2/3, or Mincl) signaling has been reported to phosphorylate and activate SHIP-2, which is able to recruit Syk to Dectin-1 or to the adaptor FcRγ, thus resulting in the activation of Syk and downstream signaling and mediate antifungal innate immune responses and Tnfα17 responses (139). In addition, the phosphatase SHIP-1 has been recently identified to co-localize with Dectin-1-phosphorylated hem-ITAM and to negatively modulate ROS production in a Dectin/Syk/P13K/PDK1/NADPH oxidase-dependent manner in response to C. albicans infection (140). Thus, a novel role of SHIP-1 in selectively controlling the balance of effectors in the Syk/P13K pathway has been identified. Phosphatase and tensin homolog deleted on chromosome 10 also serves as a negative modulator to regulate the PGE2/cAMP/PKA-signaling cascade via blocking F-actin-mediated cytoskeletal remodeling and dephosphorylating
cofilin-1 during immune defense against pathogenic *C. albicans* (141). Therefore, phosphorylation- and dephosphorylation-mediated protein kinases and phosphatases are crucial for controlling antifungal immunity.

**Ubiquitin Ligases and Deubiquitinating Enzymes in Antifungal Innate Immunity**

PTM of target proteins by polyubiquitination has been intensively studied in numerous biological systems (142–146). Seven lysine residues in ubiquitin determine the specific type of polyubiquitination. Lysine 48 (K48)-linked polyubiquitination is involved in proteasome-mediated protein degradation, whereas lysine 63-linked polyubiquitination is usually engaged in signal pathway transmission (145). In addition to K48- and K63-linked polyubiquitination, K6-, K11-, K27-, K29-, and K33-linked polyubiquitination are being exploited to address their roles in immune responses and inflammatory diseases (145). Recently, a number of studies have highlighted an important role for ubiquitination of the CLR-signaling pathway in fungal immunity (130–133).

**TRIM62**

TRIM62, also named DEAR1, is a member of the TRIM/RBCC family, which includes proteins with conserved RING finger, B-box, and coiled-coil domains (147). It has been well established that CARD9 positively modulates host immune responses following fungal infection. TRIM62 has been shown to function as a CARD9-binding component and to mediate K27-linked polyubiquitination of CARD9 at K125 to facilitate its protective role in anti-fungi immune responses (130). Similar to Card9–/– mice, Trim62–/– mice also show increased susceptibility, as well as impaired cytokine responses, in a *C. albicans* infection model (130). Therefore, TRIM62 acts as a positive regulator essential for CARD9-mediated antifungal immunity. TRIM62 or CARD9 variants are therefore potential therapeutic targets for fungal infections (Figure 2).

**Cbl-b**

Cbl-b is a member of Cbl family RING finger E3 ubiquitin ligases (142, 148). Several other groups including ours identified RING finger-type E3 ubiquitin ligase Cbl-b as a key E3 ubiquitin ligase mediating host antifungal innate immunity (131–133). The genetic deletion of Cbl-b renders mice less susceptible to systemic *C. albicans* infection, which is in line with the hyperproduction of pro-inflammatory cytokines TNF-α and IL-6, the robust release of reactive oxygen species (ROS), and improved fungal killing. At the molecular level, Cbl-b targets Dectin-2, Dectin-3, and SYK for K48-linked polyubiquitination and proteasome-mediated degradation, which further facilitates its anti-inflammatory response (131–133). Interestingly, Cbl-b small-inhibitory peptides and Cbl-b-specific siRNA provide protective efficacy against disseminated candidiasis (131, 132). Therefore, targeting Cbl-b may be a potential therapeutic strategy for disseminated candidiasis (Figure 2).

**A20**

A20 is a deubiquitination enzyme which is pivotal for tailoring innate immune responses by inhibiting the NF-kB-signaling cascade (149). IKKγ and TRAF6 activities are dampened in a non-catalytic manner by A20 (149, 150). A recent study showed that A20 is removed by autophagy, which further boosts NF-kB capacity in F4/80hi tissue-resident macrophages to facilitate the pathogen clearance during disseminated *Candida* infection (151).

**CONCLUSION**

Much progress has been made to unveil the underlying mechanisms of fungal immunity. CLRs are considered to be pivotal in orchestrating innate and adaptive immunity against fungal pathogens based on animal and some human genetic studies. The discovery of novel molecules such as Cbl-b and JNK in anti-fungi immune responses has laid the foundation for potential treatment strategies. Yet, the exact crosstalk between innate and adaptive antifungal immunity, and the yet-to-be-defined PTMs, needs to be resolved in future studies. Moreover, translational studies of newly identified molecular targets are essential for future clinical application. Thus, the studies described in this review provide direction for the rational design of therapeutic strategies in disseminated candidiasis; however, further translational studies with animal models remain to be performed before moving forward into clinical application.

**AUTHOR CONTRIBUTIONS**

JT, GL, and JZ conceptualized the scope of the review; JT, GL, and JZ wrote the review; and LT and WYL edited the review.

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