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Host cell membrane microdomains and fungal infection

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Abstract

Lipid microdomains or lipid rafts are dynamic and tightly ordered regions of the plasma membrane. In mammalian cells they are enriched in cholesterol, glycosphingolipids (GSL), GPI-anchored and signaling-related proteins. Several studies have suggested that mammalian pattern recognition receptors (PRRs) are concentrated or recruited to lipid domains during host-pathogen association to enhance the effectiveness of host effector processes. However, pathogens have also evolved strategies to exploit these domains to invade cells and survive. In fungal organisms, a complex cell wall network usually mediates the first contact with the host cells. This cell wall may contain virulence factors that interfere with the host membrane microdomains dynamics, potentially impacting the infection outcome. Indeed, the microdomain disruption can dampen fungus-host cell adhesion, phagocytosis, and cellular immune responses. Here, we provide an overview of regulatory strategies employed by pathogenic fungi to engage with and potentially subvert the lipid microdomains of host cells.

Keywords

lipid microdomains; pathogenic fungi; innate immunity

1. Introduction

The presence of lipid microdomains in the membrane was first suggested by Simon and Van Meer in MDCK cell (1988) [1]. Since then, their existence and participation in vital cellular

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processes including protein sorting, membrane trafficking, and signal-transduction events have been confirmed [2]. Lipid microdomains are enriched in sphingolipids, cholesterol and specific proteins. The lipid moiety of sphingolipids and sterols are connected through hydrogen bonds and hydrophobic interactions resulting in a highly ordered structure [2,3]. The presence of glycans covalently attached to sphingolipids can also mediate cis interactions, promoting lateral association with other glycosphingolipids and proteins [4]. The major proteins enriched in lipid microdomains include glycosylphosphatidylinositol (GPI) anchored proteins and palmitoylated proteins, making these domains organized platforms for signal transduction [5,6]. They are continuously dynamic, and upon stimulus, can coalesce in larger complexes, aggregating different raft-associated proteins and lipids in intimate association with the cytoskeleton [7]. Consequently, a number of receptors and signal transduction proteins accumulate in these regions increasing signaling efficiency. The internalization of pathogens seems to be one of the events potentially mediated by lipid microdomains [8].

The host-microbe association is a highly dynamic mechanism that involves multiple interactions. In this context, the combination of molecules gathered in the host cell platforms during the cellular response directly impacts the fate of the invaders. It is important to mention that fungal plasma membrane also contains lipid microdomains [9]. In these organisms, the microdomains have been correlated with protein sorting, cell budding and growth, biofilm formation, drug resistance, and infectivity, as extensively reviewed by Farnoud et al [9]. The presence of virulence factors, such as phospholipase B and superoxide dismutase in lipid rafts from *C. neoformans* [10] and the requirement for their integrity during the infection of macrophages by *H. capsulatum* [11] suggest that fungal lipid microdomains are also determinant components during the interaction with host cells. Nonetheless, our goal in this review is to discuss the participation of lipid microdomains from host cells during the interaction and invasion of pathogenic fungi.

2. Lipid rafts and the innate immune response to fungal infections

The major pattern recognition receptors (PRRs) involved with recognition of fungi are Toll-like receptors (TLRs) and C-type lectin receptors (CLRs) [12]. PPRs are expressed in many cell types involved in the innate immune response, and, upon activation, they trigger downstream signaling transduction pathways that lead to phagocytosis, microbial killing, and cytokine production. Among TLRs, TLR-2/1, TLR2/6, and TLR4 (surface localization) and TLR3/7 and 9 (endosomal localization) are involved in fungal responses [13–17]. Transmembrane CLRs linked to antifungal immunity include dectin-1, dectin-2, dectin-3, mannose receptor (MR), DC-SIGN, Mincle, and galectin-3 [18].

With the important exception of encapsulated pathogenic fungal species (e.g. *Cryptococcus neoformans* and *C. gattii*), the cell wall establishes the first contact of these organisms with PRRs. The precise identification of receptors to fungal pathogen-associated molecular patterns (PAMPs) is challenging since the fungal cell wall is a complex and dynamic structure [18,19], where a diversity of PAMPs, such as glucans, chitin, mannans and mannoproteins, can be exposed to phagocytes PRRs displayed by phagocytes. The cell wall

inner layer is relatively conserved among different species, but the outer layers can differ substantially and variably affect the host cell response [20].

The involvement of co-receptors during fungal recognition is associated with the amplification of the immune response. The initial mechanism of cooperation occurs during the engagement of fugal cells to their receptors, where one receptor can capture and present the fungal particle to a co-receptor [21]. In addition, the signaling response mediated after the engagement could be integrated intracellularly [22]. PRRs can cluster on the host cell surface in response to the abundance and presence of distinct ligands, strengthening the immune response. Indeed, the lack of PRRs co-stimulation seems to be the mechanism used by the dematiaceous fungus *Fonsecaea pedrosoi* to reduce the host immune response, resulting in a chronic infection called chromoblastomycosis [23]. Although leukocytes recognize *F. pedrosoi* conidia through Mincle, this association is not sufficient to promote a protective inflammatory response [23]. However, the co-stimulation with TLR agonists potentialized the inflammatory response, indicating that a collaborative process involving distinct PRRs is required to induce a protective response. Remarkably, the administration of LPS to mice infected with *F. pedrosoi* substantially reduced the fungal burden. In addition, fungal burden decrease was also observed in mice infected subcutaneously with *F. conidia* and topically treated with Imiquimod, a synthetic agonist for TLR7. This imidazoquinolide derivative was successfully used in human chromoblastomycosis [24]. The raft environment seems to be a distinct place where paired receptors could cooperate and amplify the cellular response. Indeed, the recruitment of TLRs to these domains after ligand engagement has been reported [25].

All TLRs, except TLR3, lead to the recruitment of the adaptor molecule MyD88 [26]. MyD88 contains a death domain (DD) at the N terminus that interacts with the kinase IRAK, forming the Myddosome complex, a platform that leads to transcriptional activation through NF-κB and AP-1. TLR4 clustering in lipid rafts produces a large oligomeric assembly of MyD88 with IRAK-1, which is associated with enhanced activation [27]. The importance of rafts for MyD88-dependent TLR activation has been demonstrated in macrophages genetically modified expressing high lipid raft content [28]. These cells displayed a remarkable content of TLR4 in lipid raft regions and were more sensitive to TLR2, 7, and 9, but not to TLR3 stimulation. Other PRRs can collaborate with TLRs, including dectin-1, DC-SIGN, and the scavenger receptor (CD36) [29–32]. Their association could be linked with TLR recruitment to lipid rafts, amplifying the response. For instance, CD36 is a palmitoylated protein usually enriched in lipid rafts, functioning as TLR2 collaborator within the domains [29]. Similarly, CD14 is a GPI-anchored protein present in raft, and its association with LPS stimulates the translocation of TLR4 into lipid microdomains [33,34]. Numerous studies have demonstrated that the association between TLR2, 4, 5, 7 or 9 and dectin-1 strengthens the immune response to achieve appropriate biological responses to pathogenic fungi [22,31,35,36]. Confirming that PRRs collaboration is important to an effective immune response Gantner and colleagues have shown that activation of TLR2 by zymosan particles is not sufficient to induce high levels of reactive oxygen species (ROS) production in macrophages, which requires a collaboration with dectin-1 [35]. In contrast with TLRs, which is able to recognize soluble microbial components, dectin-1 becomes activated only with immobilized forms of β-(1,3)-

glucan, leading to a “phagocytic synapse” and a robust antifungal response [37]. The fact that dectin-1 translocates into lipid microdomains of mouse bone marrow-derived DC in response to zymosan stimuli suggests that clustering of these heterologous PRRs takes place in these platforms [38].

The crosstalk between PRRs expressed by DCs has an additional importance for orchestrating the differentiation of T helper (Th) cells. Dectin-1 activation drives DCs maturation and priming of naive CD4 T cells towards Th1 and Th17 phenotypes, commonly involved with protection against pathogenic fungi [39]. Translocation of dectin-1 into lipid microdomains is essential for Syk phosphorylation and consequently the signal transduction until the nuclear factors [38]. Additional evidence shows that the enrichment of dectin-1 and Syk phosphorylation in zymosan contact sites require excluding the inhibitory signal of the phosphatases CD45 and CD148, which strongly reinforces the importance of the membrane domains architecture in immune signaling pathway against fungi [37].

3. Glycosphingolipids-enriched lipid rafts as a platform for fungal recognition.

Glycosphingolipids (GSLs) are a heterogeneous subclass of glycolipids [4]. Lactosylceramide (LacCer) is a remarkable GSL that together with PRRs mediate the immune response to pathogenic fungi. Yeasts species such as *C. neoformans*, *Candida albicans, S. cerevisiae*, and the yeast-like *Pneumocystis jirovecii* as well as the dimorphic fungi *Histoplasma capsulatum, P. brasiliensis*, and *Sporothrix schenckii* directly bind to this domain (Fig. 1) [40]. The presence of galactose as a terminal residue of LacCer is linked to β-glucan recognition [41]. LacCer is predominantly increased in human glioma brain cells, and functions as binding sites for *C. neoformans* [40]. Furthermore, LacCer is enriched in lipid rafts of human neutrophils and their association with β-glucan induces migration, phagocytosis and superoxide generation (Fig. 1) [42–44]. The presence of a β–1,6-long glucosyl side-chain in the β-glucan seems to be crucial for Lyn and PI3K activation and neutrophil migration [45].

LacCer enriched domains could still interact with immune receptors, such as CR3, a complement receptor composed of integrins CD11b (Mac-1) and CD18 [41]. CR3 co-localizes with LacCer-enriched membrane microdomains when human neutrophils are activated with the CD11b monoclonal antibody VIM12 [46]. The signaling transmitted by the stimulation is mediated through the interaction of LacCer and the C-terminal portion of CD18, which leads to Lyn phosphorylation (Fig. 1).

4. Role of lipid rafts in fungal infection.

In this section, we will review studies that have focused on the contribution of lipid microdomains to host interaction with the following fungal pathogens: *C. albicans*, *C. neoformans*, *Pneumocystis jirovecii, Aspergillus fumigatus, P. brasiliensis*, and *H. capsulatum* (for a brief summary of molecular interactions involving host cell lipid rafts, see Table 1).

4.1 Candida albicans

One of the common strategies to investigate the role of lipid rafts in PRRs crosstalk is the disruption of host cell lipid domains using methyl-β-cyclodextrin (m-β-CD) and Deoxycholate Amphotericin B (DAmb), drugs that extract and immobilize, respectively, sterols from the plasma membrane [60]. Although DAmb and other compounds that immobilize membrane sterol in mammalian cells have been extensively used in lipid domains studies, we cannot rule out their steric interference that could impact fungal binding. Pre-treatment of monocytes with both drugs directly affected *C. albicans* engulfment, reducing lipid raft coalescence and dectin-1 recruitment to *C. albicans* binding sites (Fig. 2) [47]. However, treatment of host cells with m-β–CD did not prevent the production of cytokines, indicating that the co-participation of non-raft PRRs could bypass the correct lipid raft assembly and trigger the stimulus required for cytokine synthesis. In addition, raft disruption on host cells drastically dampened Th17 and Th1 responses, suggesting that the preservation of dectin-1 dynamics in lipid-raft of antigen presenting cells is crucial for specific T-cell responses in this model [47]. Thus, although the recognition of *C. albicans* by human monocytes takes place independently of the integrity of lipid microdomains, a correct and balanced response by these leukocytes rely on the membrane organization provided by the rafts. Further studies are necessary to investigate the impact of host lipid domains during interaction with *C. albicans* in other cells from the immune system.

4.2 Cryptococcus neoformans

A set of studies demonstrates that *C. neoformans* penetration into human BMEC (HBMEC) is a lipid raft-dependent process (Fig. 1) [48–50]. Adhesion to HBMEC seems to be mediated by hyaluronic acid (HA), a fibrous structure outside the *C. neoformans* cell wall [61,62]. Treatment with hyaluronidase and deletion of *CPS1*, the coding gene of hyaluronic synthase, significantly reduced the binding of *C. neoformans* to HBMEC [61]. In addition, *C. neoformans* HA is recognized by CD44 at the HBMEC surface, a step blocked by anti-CD44 antibodies and CD44 shRNA treatments [62]. Association of *C. neoformans* with HBMEC induces the redistribution of CD44 to lipid rafts at the binding sites. Remarkably, treatment of host cells with filipin, a polyene antibiotic that immobilizes and reduces sterol availability, impacting the lateral mobility of host cell membranes, significantly decreases yeast association with HBMEC, suggesting that lipid raft integrity is required.

The colocalization of CD44 with caveolin-1 (Cav-1) also supports the role of these platforms during the internalization of *C. neoformans* by HBMEC [48,50]. Cav-1 is the main component of caveolae, a subset of microdomains involved with signal transduction and endocytosis (Fig. 1) [63]. The anchorage of caveolae on the plasma membrane is sustained by the connection of Cav-1 with actin cytoskeleton [64]. Thus, caveolae-dependent endocytosis is regulated through reorganization of the actin cytoskeleton, and triggered by phosphorylation of Cav-1 [65]. When Cav-1 expression is down-regulated in HBMEC, the number of internalized yeasts is reduced, with a small impact on fungal adhesion, suggesting that Cav-1 is not involved with binding but has a strong impact in the fungus' internalization [50]. The binding of *C. neoformans* and HA to HBMEC also induces Cav-1 phosphorylation, a change that has been linked to caveolae-mediated internalization [50].

Cytoskeleton rearrangement was further investigated during *C. neoformans* internalization. Inhibition of actin polymerization and tyrosine kinase activity reduced the association and blood-brain barrier (BBB) transmigration of *C. neoformans*, confirming that both events are required for invasion [66]. Actin rearrangement appears to be initiated by *C. neoformans'* binding to receptors at the endothelial plasma membrane, triggering the activation of Rho GTPases (RhoA, Rac1, and Cdc42) and phosphorylation of focal adhesion kinase (FAK), protein kinase C α (PKCα), and ezrin, promoting yeast transmigration across the BBB (Fig. 1) [66].

A pre-adhesion step could be mediated by extracellular vesicles (EVs) released by *C. neoformans* (Fig. 1) [51]. EVs are lipid bilayer membrane compartments used by cells to export a number of molecules, including proteins, polysaccharides, pigments, lipids and nucleic acids [67]. Their participation during disease development has been suggested for different fungi. Treatment of HBMEC with *C. neoformans*-derived EVs induces membrane ruffling, a response usually caused by cytoskeletal rearrangement [51]. These results were also observed when *C. neoformans* or HA were incubated with HBMEC [48]. However, the response mediated by EVs is CD44-independent [51]. Incubation with EVs promoted the redistribution of lipid raft components recruiting CD44, Cav-1, and up-shifted the intracellular distribution of β-actin. Changes induced by EVs could enhance *C. neoformans* recognition and transcytosis. Corroborating with the cellular changes, intravenous administration of EVs enhanced the ability of *C. neoformans* to reach the brain in a murine mice model of cryptococcosis.

Finally, the interplay between HIV infection and invasion of *C. neoformans* into HBMECs seems to be also mediated by lipid rafts [68,69]. It has been reported that a specific domain of the glycoprotein 41 (gp41), induces the recruitment of CD44 to HBMECs lipid rafts, and potentiate the *C. neoformans* invasion. The protein gp41 is a transmembrane subunit of the HIV envelope protein complex that mediates virus fusion [70]. The recombinant peptide, encompassing the specific sequence of amino acids of gp41 (between 579 and 611), was able to stimulate *C. neoformans* invasion *in vitro* and its intravenous injection enhanced brain infection in mice by *C. neoformans* [68]. In addition, treatment with gp41-I90 and *C. neoformans* synergistically enhanced the transmigration of monocytes in HBMEC and *in vivo* [71]. These findings contribute novel perspectives about the role that lipid rafts play in comorbidity between HIV and fungal neuro-infection.

4.3 Pneumocystis jirovecii

A balanced inflammatory response in pneymocystosis (PcP) is crucial for a patient to resolve *Pneumocystis* infection [72]. Lipid rafts are involved in the response of alveolar epithelial cell (AEC) to the β-glucans of *Pneumocystis* (PCBG). LacCer was responsible for stimulating the release of MIP-2 from AEC in a process dependent of PKC [52,53]. Treatment of AECs with monoclonal antibodies against LacCer, but not GM1, impairs internalization of PCBG [53]. In DCs, PCBG stimulates IL-23 and IL-6 secretion, culminating in the activation of the IL-23/IL-17 axis [54]. This activation is lipid raft-mediated, and dectin-1 is the main receptor, leading to the downstream activation of Syk and NF-κB. However, there is evidence that suggests that LacCer works as a co-receptor in this

process, as β-glucan stimulation acts to mobilize and concentrate LacCer within the lipid microdomains [54]. These findings are clinically relevant because these cytokines are crucial for a Th17 phenotype, which plays a major role in fungal clearance and defense [73,74].

Overall, the major problem in patients with PcP is the overstimulation of the inflammatory response, which is induced by the interaction of host cells and fungal β-glucans [75]. Interestingly, an array of studies show that this response is significantly dampened by the inhibition of host cells lipid rafts [54,76], not only evidencing their role in the pathology but also suggesting that they could be a novel therapeutic target to pharmacological agents such as nystatin, which is capable of inhibiting the assembly of glycosphingolipid-cholesterol rich microdomains in host cells, interrupting PCBG recognition and potentially impairing fungal internalization [52].

4.4 Aspergillus fumigatus

For airborne pathogens, AECs and resident alveolar macrophages are the first cells involved with fungal recognition in the lung [77]. Recently, the ability of bronchial epithelial cells to attenuate virulence of *A. fumigatus* conidia was also linked to a mechanism of internalization involving lipid rafts [78]. The presence of flotillin-2 and caveolin in a ring-like structure surrounding internalized conidia, along with other host cell proteins, were demonstrated. Similar to Cav-1, flotillin-2 is also a protein enriched in lipid rafts and associated with endocytosis [78]. Remarkably, treatment of host cells with filipin reversed the ability of bronchial epithelial cells to decrease the conidia's virulence [78].

The pigment melanin is an important virulence factor that protects fungal cells against host oxidative responses [79]. A recent study using a melanized-wild type conidia and a melanin-free conidia (*pksP* mutant) suggested that the presence of this pigment also helps *A. fumigatus* to control lipid rafts' composition at the initial steps of fungal internalization and during the phagolysosomal maturation [55]. Remarkably, GM1 and flotillin-1 were significatively reduced in the phagolysosomal membranes containing melanized conidia. Since the recruitment of the proton pump V-ATPase is dependent on the presence of flotillin-1, a substantial reduction on phagolysosome acidification was observed, allowing conidia germination [55,80]. Phagosome maturation involves gradual changes to membrane composition, including the assembly of subunits of V-ATPase, and NADPH oxidase [81]. In line with that, NADPH oxidase complex assembly at flotillin-containing membrane microdomains was also compromised by melanin [55]. The signaling pathway that triggers this cascade of events is not fully elucidated, but the same study shows that formation of lipid rafts at the phagolysosomal membrane is dependent on calmodulin (CaM) activity. In this context, *A. fumigatus'* melanin mediates Ca^{2+} sequestration inside the phagosome lumen, which reduces the availability of these ions in the peri-phagosomal area where calmodulin activation takes place [55,82]. Accordingly, treatment with calmodulin inhibitors significantly reduced the co-localization of raft marker the ganglioside GM1 in phagolysosomes containing conidia [55].

The role of melanin is complex, and can up or downregulate fungal phagocytosis depending on its structure [79]. An anti-phagocytic role was observed for melanin in *A. fumigatus* conidia, and this was correlated to modulation of flotillin-associated microdomains [55].

The *pksP* mutant conidia were more phagocytosed by macrophages, but in the absence of flotillin, the phagocytosis of this mutant was reduced. The presence of melanin and a hydrophobin rodlet at conidia cell wall prevents immune recognition [20]. However, the *pksP* conidia expose β-glucan, which activates LC3-associated phagocytosis (LAP), promoting fungal killing through NADPH oxidase activity in the phagosome [82]. LAP activation was recently shown to enhance the antimicrobial activity in macrophages and DCs infected by *C. albicans, A. fumigatus* or *H. capsulatum*, but the association between LAP and membrane microdomains has not been evaluated [82,83]. Purified melanin inhibits NADPH oxidase activity through exclusion of its subunit p22phox from the phagosome membrane [82]. Indeed, it is important to note that inhibition of Ca^{2+}–CaM signaling by fungal melanin was also correlated with the blocking of LC3 recruitment to the phagosomal membrane of macrophages, which suggests a possible role of membrane microdomains in phagocytic routes involved with phagolysosomal activity [84].

4.5 Paracoccidioides brasiliensis

P. brasiliensis is a thermally dimorphic fungus, and infection is initiated by the inhalation of conidia or arthroconidia that differentiate to yeasts in the host's lung [85]. Incubation of human AEC line A549 with yeasts of *P. brasiliensis* showed an enrichment of the raft marker GM1 at the fungal-host cell adhesion points. Binding was followed by a rapid activation of SRC family kinase (SFK) and ERK1/2. Treatment of A549 cells with nystatin and m-β-CD resulted in reduced fungal association and impaired activation of AECs. The lipid raft composition analysis confirmed that the association of *P. brasiliensis* yeasts with A549 cells induced the phosphorylation of SFK in lipid rafts. Pre-treatment with m-β-CD also abolished activation of SFK. Further studies demonstrated that *P. brasiliensis* recognition by A549 also promotes an upregulation of α3 and α5 integrins. Both integrins were recruited to caveolin-associated lipid rafts at the *P. brasiliensis*-A549 point of contact, suggesting the involvement of caveolae in fungal endocytosis [56]. In fact, treatment of A549 cells with genistein, a tyrosine kinase inhibitor used to block caveolae-dependent endocytosis, inhibited the adhesion and invasion of the fungus, demonstrating the importance of caveolae domains on the internalization of *P. brasiliensis* [86]. The role of AEC in driving an inflammatory response to an invasive fungal infection has been addressed and linked to a correct raft assembly [87,88]. The recruitment of α3 and α5 integrins to these domains induces IL-6 and IL-8 secretion by A549 cells [56]. IL-8 release by epithelial cells is essential for neutrophil recruitment and inflammatory response orchestration [89].

The enrichment of GM1 at *P. brasiliensis* binding sites in A549 cells and the previous studies showing that other fungi recognize LacCer at the plasma membrane of neutrophils suggest that this pathogen could bind to other GSL at the host cell surface. In fact, GalCer, LacCer, CTH (Galα1-4Galα1-4Glcα1-1Cer), GD3, GM1, and GD1a supported *P. brasiliensis* adhesion [90]. In addition, pre-incubation of *P. brasiliensis* yeast with GM1 made these cells reactive to the GM1-glycan ligand cholerae toxin subunit B (CTxB). Steric blocking of GM1 with CTxB, and GM3 with the antibody DH2, significantly reduced the adhesion of *P. brasiliensis* to human lung fibroblasts, confirming that these GSL could support fungal binding [90].

4.6 Histoplasma capsulatum

H. capsulatum is another thermally dimorphic fungus that affects primarily the lungs. The mechanism of interaction of yeasts with AEC is similar to described for *P. brasiliensis*. Translocation of α3 and α5 integrins and signaling machinery to lipid domains are associated with secretion of IL-6 and IL-8 by these cells. However, the role of AEC in fungal control remains elusive [57]. On the other hand, infection of macrophages is known as a critical step during histoplasmosis development. In some virulent strains of *H. capsulatum*, yeasts have the ability to block macrophage activation by inhibiting the recognition of the β-(1,3)-glucan by dectin-1 [91].

The role of PRRs on the innate immune response to *H. capsulatum* yeast has been a focus of several studies [92–94]. *H. capsulatum* yeasts use the β2 integrin (CD18) of CR3 to mediate recognition by macrophages through the surface exposure of fungal 60 kDa heat shock protein (HSP60) [95]. CR3 might be the exclusive phagocytic receptor involved in the internalization of this fungus (non-opsonized) by macrophages suggesting that a phagocytic synapse involving other PRRs at the lipid membrane domain is not required during host cell invasion [92]. However, when sialic acids are removed from host cells by enzymatic treatment, an impairment of macrophage association with *H. capsulatum* was observed. These results indicate that sialylated GSL could be involved in fungal interaction with immune cells [92]. Results from our group suggest that GSLs are involved in lipid rafts organization during macrophage infection by *H. capsulatum* (Fig. 1) [58]. We demonstrated the enrichment of cholesterol at the binding sites of *H. capsulatum* to macrophages (Fig 2). Depletion of cholesterol after treatment of macrophages with m-β-CD reduces *H. capsulatum* adhesion and internalization. Since m-β-CD can also extract other host membrane components it is important to confirm that the PRRs or their co-receptors are still at regular levels [60]. In addition, peritoneal macrophages from mice deficient for complex gangliosides production, such as GM1 and GD1a (*B4galnt1*$^{-/}$) have a reduced ability to recognize *H. capsulatum* [58]. GM1-associated microdomains appear to be extremely important for *H. capsulatum'* adhesion to host cells, although not to opsonized yeast cells. Our data also suggest an interesting interplay between GM1 and the CD11b/CD18 integrin. We demonstrated that both GM1 and the CD18/CD11b integrin are recruited to the macrophage/*H. capsulatum* interaction site. Furthermore, CD18 recruitment to lipid microdomains fractions was reduced in macrophages from *B4galnt1*$^{-/-}$, indicating that GM1 could be an accessory molecule that facilitates the recruitment of CR3 into macrophage lipid rafts [58]. Remarkably, macrophage-*H. capsulatum* association induced recruitment of laterally organized lipid domains first at the site of fungus–phagocyte contact and then along the entire surface of the host cell.

As previously mentioned, the CR3 pathway involves lipid microdomains, and the signaling is mediated by LacCer-associated Lyn that activates cytoskeleton rearrangement [96]. HSP60 is expressed on the fungal surface in clusters, which may facilitate the CR3 binding process [95]. However, CR3 contains multiple binding sites, including a lectin site at the C-terminal domain that recognizes β-glucans [97]. *H. capsulatum* lacking α-(1,3)-glucan induces activation of the dectin-1 signaling pathway in macrophages, and the colocalization of dectin-1 and CR3 into GM1 microdomains was observed at the cell interface of

interaction between host cells and heat-killed yeasts (Fig. 1) [59]. The collaborative work between dectin-1 and CR3 into lipid raft was crucial to trigger the cytokine response through downstream signaling of Syk, JNK, and transcriptional activity of AP-1 [59]. These results collectively suggest that *H. capsulatum* yeasts can avoid the recruitment of specific receptors to the space of lipids rafts in the plasma membrane of macrophages to more effectively facilitate the yeasts' infection of macrophages.

5. Conclusion

Understanding lipid raft assembly during fungal-host cell interaction is an interesting paradigm that reveals other mechanisms by which each fungus can modify the host cell response and promote a variety of clinical conditions. It is a multistep process that impacts changes in the whole plasma membrane and, although the players are well-known, the dynamics behind the molecular events remain largely opaque. Because these pathways are tightly involved in fungal recognition and its intracellular fate and the elaboration of immune responses, they pay pose as attractive targets for therapeutics and pharmacological management of mycoses.

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Data availability statement:

The data that support the findings of this study (displayed at Figure 2) are openly available in https://doi.org/10.1371/journal.pone.0142531.g003 and at http://doi.org/[10.1371/journal.pone.0142531] and http://doi.org/10.1111/cmi.12976, reference numbers [47, 58].

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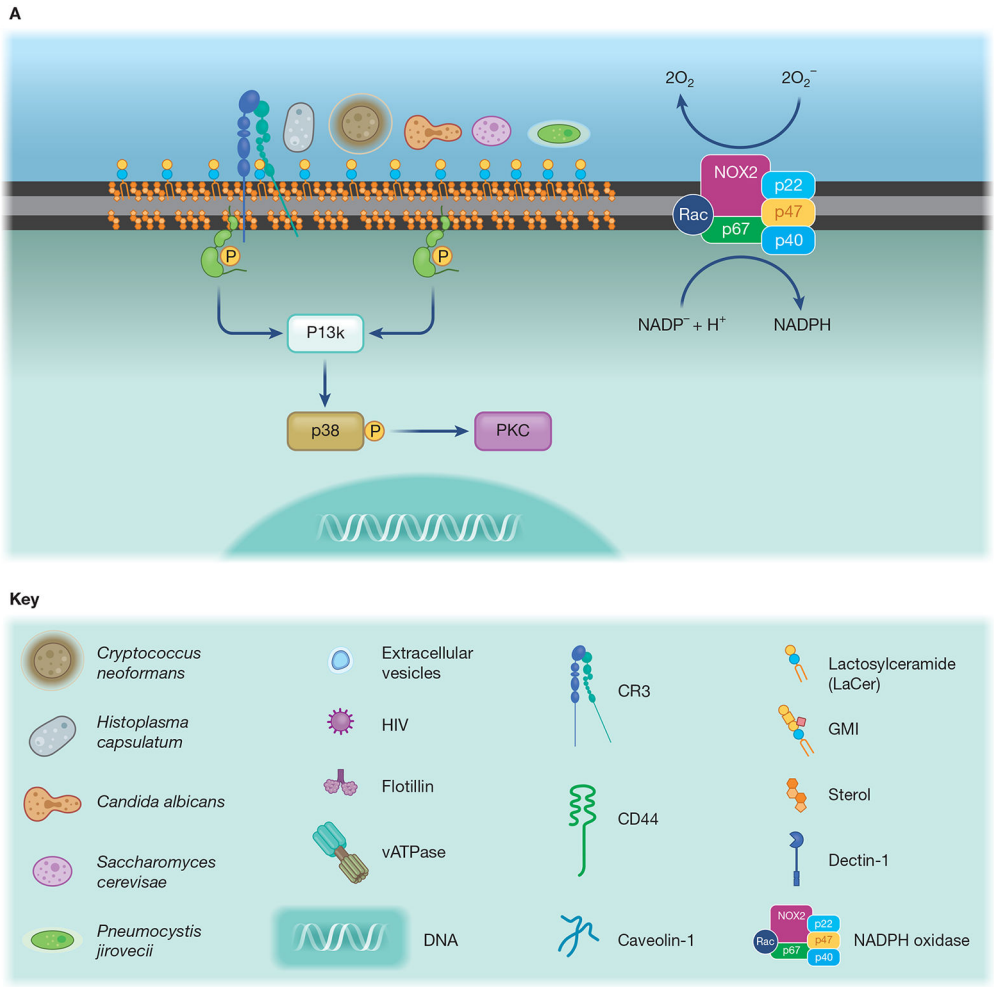
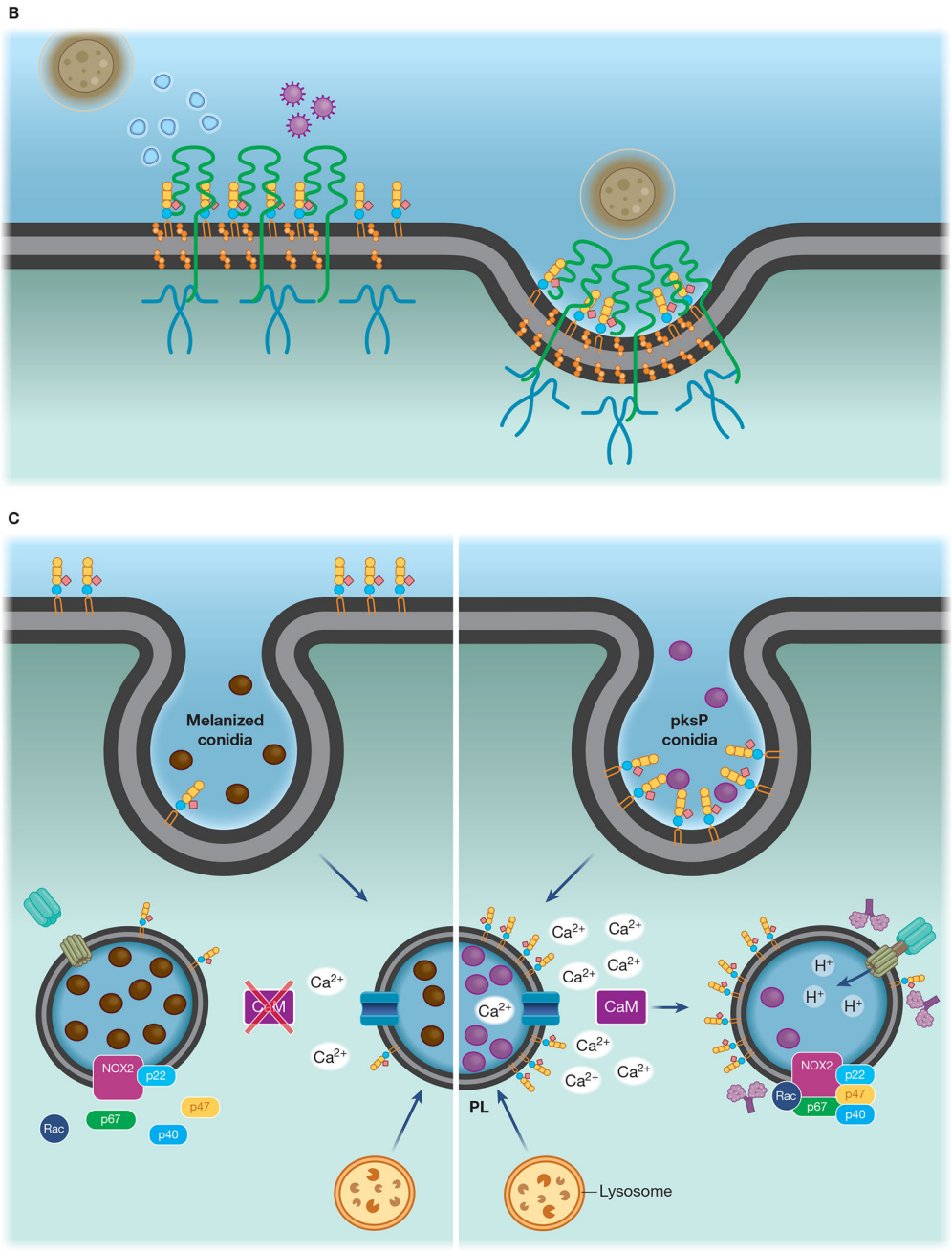
Take Away

- Lipid microdomains are ordered regions of the plasma membrane enriched in cholesterol, glycosphingolipids (GSL), GPI-anchored and signaling-related proteins.
- Pathogen recognition by host immune cells can involve lipid microdomain participation. During this process these domains can coalesce in larger complexes recruiting receptors and signaling proteins, significantly increasing their signaling abilities.
- The antifungal innate immune response is mediated by the engagement of pathogen-associated molecular patterns to pattern recognition receptors (PRRs) at the plasma membrane of innate immune cells. Lipid microdomains can concentrate or recruit PRRs during host cell-fungi association through a multi-interactive mechanism. This association can enhance the effectiveness of host effector processes. However, virulence factors at the fungal cell surface and extracellular vesicles can re-assembly these domains, compromising the downstream signaling and favoring the disease development.
- Lipid microdomains are therefore very attractive targets for novel drugs to combat fungal infections.





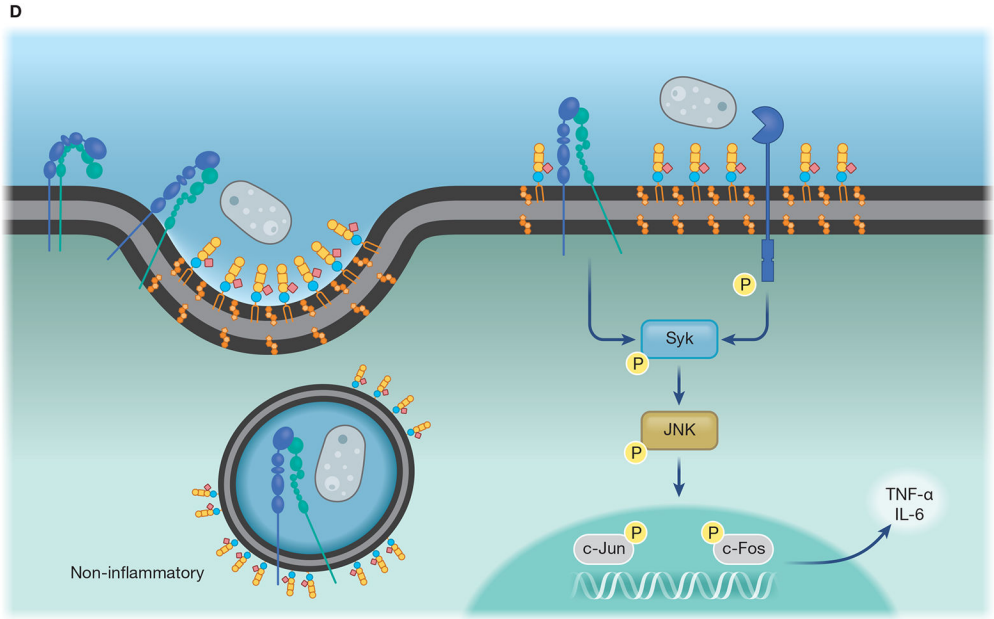


Figure 1- Interplay of fungal pathogens and host cell lipid microdomains.

(A) Lipid microdomains enriched in LacCer are binding sites for fungal β-glucan and a signaling platform for CR3 recruitment, allowing this receptor to respond to non-opsonized pathogenic fungi. LacCer domains trigger a signaling pathway through Lyn, PI3K, p38, and PKC, leading to actin polymerization, required for migration, phagocytosis, and ROS production. (B) Caveolae is involved in the invasion of *C. neoformans* yeast in HBMEC. CD44 is located in caveolin-1-enriched membranes (Cav-1), where it recognizes the fungal hyaluronic acid. The intimate contact between Cav-1 and actin promotes the fungal internalization by HBMEC. The activation of Rho GTPase is correlated with actin remodeling and increased fungal internalization, which could be mediated by Cav-1. In addition, yeast extracellular vesicles and the HIV gp41 stimulate the clustering of CD44 on Cav-1 domain, increasing binding and invasion of *C. neoformans* yeast. (C) Flotillin domains on phagolysosomal membrane of macrophages are affected by melanin from *A. fumigatus* conidia. In comparison to non-melanized conidia (pksP), WT conidia block the release of calcium ions from the phagolysosome lumen to the cytoplasm, avoiding the activation of calmodulin (CaM). Conidia melanin impairs the formation of flotillin domains on phagolysosomal membranes and reduces the recruitment and assembly of V-ATPase and NADPH. (D) Recruitment of CR3, the main macrophage receptor for *H. capsulatum*, to lipid microdomains requires GM1. When β-(1,3)-glucans are exposed on the *H. capsulatum* cell wall the lipid microdomains allow the cooperation between CR3 and dectin-1, enhancing the inflammatory signaling through Syk, JNK, AP-1 (c-Jun/c-Fos).

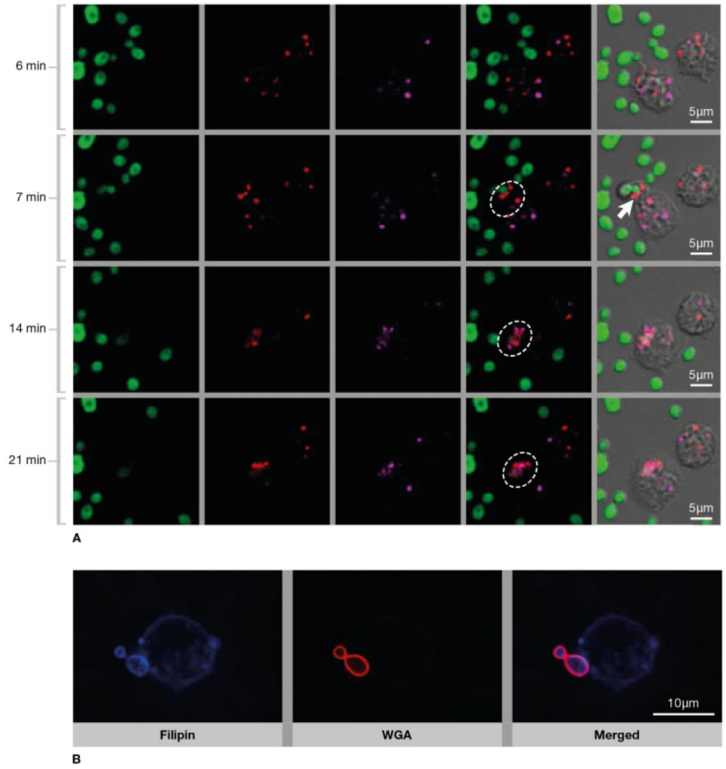


Figure 2- Lipid domains from host cells are involved with association of fungal organisms.
(A) Involvement of lipid rafts in Dectin-1-mediated phagocytosis of *C. albicans* (Ca). Time lapse analysis of Dectin-1 (red) and lipid raft components (cholerae toxin B/GM1, magenta) during the initial steps of association between *C. albicans* (green) and human monocytes. The timescale shows the recruitment of the glycosphingolipid GM1 and the PRR dectin-1 to the point of monocyte-fungal cell contact (white arrow and dashed circles). Scale bars, 5 µm. Adapted from Turris et al [47]. (B) Importance of lipid rafts in the association of *Histoplasma capsulatum* (Hc) with macrophages. Filipin staining (cholesterol/blue) shows the enrichment of sterol on the adhesion area indicating that cholesterol within lipid microdomains could be involved in Hc- macrophages recognition. Merged images denoting

the co-localization of filipin staining and WGA-Alexa546-Hc (red). Bar, 10 μm. Obtained from Guimarães et al [58).

Table 1:

Examples of interaction between host lipid microdomains and pathogenic fungi

Fungal pathogen	Host cell	Molecular Interaction (pathogen/ host)	Functional Response	References
Candida albicans	Monocytes	β- (1,3)- glucan	Phagocytosis	[47]
	Neutrophils	β-(1,6)-side chain-branched β-glucan	Chemotaxis	[45]
		HA[1]		[48][49][50]
Cryptococcus neoformans	HBMEC[1]	EVs[1]	Adhesion and brain invasion	[51]
	AEC[1]	β- (1,3)- glucan	MIP-2 synthesis[1]	[52][53]
Pneumocystis jirovecii	Human dendritic cells	β- (1,3)- glucan	Activation of the IL-23/IL-17 axis	[54]
Aspergillus fumigatus	Macrophages	DHN[1] melanin	Inhibition of acidification of PL[1]	[55]
Paracoccidioides brasiliensis	AEC	Not determined	IL-6 and IL-8 secretion	[56]
Histoplasma	AEC	Not determined	IL-6 and IL-8 secretion	[57]
capsulatum	Macrophages	HSP60[*] and β- (1,3)- glucan[*]	Phagocytosis and cytokine synthesis	[58][59]

[1]HA – Hyaluronic acid, HBMEC - Brain microvascular endothelial cells; EVs - Extracellular Vesicles; DHN - Dihydroxynaphthalene; PL – Phagolysosome; AEC – Alveolar Epithelial Cells, MIP-2 - Macrophage inflammatory protein-2, HSP60 - Heat Shock Protein 60.

[*]possible ligands according to literature findings