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The Intracellular Life of *Cryptococcus neoformans*

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Abstract

Cryptococcus neoformans is a fungal pathogen with worldwide distribution. Serological studies of human populations show a high prevalence of human infection, which rarely progresses to disease in immunocompetent hosts. However, decreased host immunity places individuals at high risk for cryptococcal disease. The disease can result from acute infection or reactivation of latent infection, in which yeasts within granulomas and host macrophages emerge to cause disease. In this review, we summarize what is known about the cellular recognition, ingestion, and killing of *C. neoformans* and discuss the unique and remarkable features of its intracellular life, including the proposed mechanisms for fungal persistence and killing in phagocytic cells.

Keywords

granuloma; Trojan horse; disease tolerance; fungal immunity; intracellular pathogen; *C. neoformans* killing

Introduction to The Biology of *Cryptococcus Neoformans*

Environmental Organism and Treatment of Disease

Cryptococcus neoformans was first described in 1894 by Otto Busse, when the organism was recovered from a lesion in a woman's tibia (1). The pathogenic yeast can be found worldwide in several environmental niches and has been isolated from soil, trees, and animals, in particular from avian guano (1, 2). Exposure to *C. neoformans* does not usually lead to overt disease, and epidemiological data led to the accepted view that establishment of an asymptomatic latent state may be the most common outcome of infection (3–5). Even from the early clinical cases described, an association between cryptococcosis and immunosuppression was already inferred (39, 40). In fact, in immunosuppressed patients,

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reactivation of infection is frequently fatal. Patients develop pneumonia and meningoencephalitis, and brain involvement predicts high mortality and morbidity, even with aggressive antifungal drug therapy (6).

Immunity to Cryptococcosis

Serological studies show that 80% of children in urban environments have been infected with *C. neoformans*, without any discernible clinical manifestations (4, 7). Primary infection most likely occurs via inhalation of spores or desiccated yeast cells from environmental sources. The physical characteristics of these infectious particles, such as size and capacity to become airborne, allow deposition in the lungs (8). There, the yeast particles encounter an alveolar macrophage or dendritic cell and trigger an immune response, culminating in sterilization or, most likely, restriction of infection within a granuloma. The resulting granulomas are usually well circumscribed, self-limited, and benign (Figure 1) and are composed mainly of mature mononuclear phagocytes, histiocytes, and giant multinucleated cells enveloping the yeast cells (9).

Efficient control of *C. neoformans* requires a delicate balance of both Th1- and Th2-type responses (10–12). Depletion of cytokines by genetic disruption or antibody neutralization has confirmed that a Th1-type response is essential to control infection; these studies are summarized in Table 1. In fact, mouse strains show differential susceptibilities that correlate with a stronger Th1 versus Th2 skewing (13) and with the presence of complement cascade member C5 (14). Depletion of Th1-type cytokines, such as interferon-γ (IFN-γ) and interleukin (IL)-12, consistently results in decreased mouse survival (15, 16), whereas loss of hallmark Th2-type cytokines increases mouse survival (17). In these models, Th1 or Th2 cytokine bias is reflected in both granuloma composition and control of fungal burden (18). Although a predominantly Th1-type response results in mouse survival, too strong of a Th1-type polarization cannot prevent brain dissemination (19–22) and associated mortality, and the Th2 component is required for the most efficient immune response. Although an impressive body of work has been carried out to characterize cytokine dependence, an understanding of immunity to cryptococcosis is still incomplete. For example, lack of the Th1 major cytokine tumor necrosis factor α (TNF-α) did not influence mouse survival, but administration of TNF-α was beneficial (23). As another example, Th17 immunity was crucial for *Candida albicans* mucosal immunity (24) but appears to play a lesser role in cryptococcal disease: In models of cryptococcosis, deletion of Th17-type responses did not influence the outcome of primary infection or the efficiency of vaccination (25).

Macrophages are crucial for control of cryptococcosis, as evidenced by the observation that depletion of host macrophages and dendritic cells results in dramatically reduced survival after *C. neoformans* challenge (26, 27). Two studies of the effects of macrophage depletion on lung fungal burden produced contradictory results (27, 28); however, both studies demonstrated that mouse macrophages require a particular activation profile to become fungicidal (28). Macrophages with a mixed classical and alternative activation phenotype are seen during experimental models of cryptococcosis (19). Although they are less studied, other types of innate immune cells are found in granulomas and may play a role in defense against cryptococcosis (29). The presence of either excess eosinophils or excess neutrophils

is associated with poor control of infection in mice (30, 31), whereas eosinophils might have a beneficial role in rats (32).

An extensive body of literature shows that induced or passively administered antibodies can mediate significant protection from cryptococcosis (33). However, the role of humoral immunity in the cryptococcosis model is not adequately explained by classical mechanisms of antibody-mediated immunity, which has led to the discovery of novel immunoregulatory functions of antibodies (33).

Various investigators have addressed the immunological mechanism for effective immunization against *C. neoformans* challenge (3, 27, 34, 35). For example, immunization with capsular mannoproteins was able to prolong mouse survival (34). An alternative approach was to design an IFN-γ-producing *C. neoformans* (IFN-γ is a strong Th1-type cytokine) (25, 36–38). This strategy resulted in complete protection from a posterior challenge, accompanied by a Th1-biased lung cytokine pattern, classical activation of macrophages, and increased production of nitric oxide (NO) (37), and demonstrated how appropriate manipulation of the host immune system, in particular macrophage activation, can be an effective therapeutic option. At this time, there is a reasonable consensus that defense against cryptococcosis depends on an appropriate collaboration of Th1 cells with macrophages.

Evidence That Intracellular Residence Contributes to Virulence and Immune Escape

Evidence from Pathological Studies

C. neoformans lesions in autopsies (9, 39–41) and experimental models (42) show fungal cells inside granulomas, known as cryptococcomas (Figure 1b,c). Cryptococcal granulomas are less inflammatory than *Mycobacterium tuberculosis* granulomas, suggesting a dormant and controlled infection. In well-organized granulomas the yeast is localized within the cytosol of giant cells or macrophages, but in the absence of granulomas yeasts are both intracellular and extracellular (Figure 1f) (41). Neutrophilic infiltrates are not common in human cryptococcal lesions, whereas $CD4^+$ T cells are found in immunocompetent patients.

In rats (42), mice (3, 43), and rabbits (44), *C. neoformans* can be found associated with lung macrophages, in some cases for months, without obvious clinical manifestations. In mice, *C. neoformans* is rapidly ingested by phagocytes, and in one model of experimental infection, there was a fluctuation in intracellular and extracellular residence during the first 24 h (43). At day 7, a shift occurred toward the intracellular lifestyle, coincident with formation of granulomas. At day 28, most yeast cells were found within multinucleated giant cells, as illustrated in Figure 1e. In this model, the budding index was higher for intracellular than for extracellular *C. neoformans*, sparking the hypothesis that intracellular residency is favorable for *C. neoformans* growth. Hence, both early infection and long-term persistence find *C. neoformans* cells associated with host macrophages, supporting the importance of intracellular residence within them (43).

Evidence from Animal Models

Animal models have found evidence consistent with the view that fungal residency within macrophages contains the infection while allowing the fungus to persist in tissue. Rats are more resistant than mice are to *C. neoformans* infection, but the two rodent systems have provided complementary information. Rats' superior resistance to cryptococcosis is associated with a more effective macrophage fungicidal capacity, an effect attributed to increased production by macrophages of lysozyme and reactive oxygen species (ROS) (28, 42). Similar to the situation in mice, *C. neoformans* resistance in rats is associated with a strong Th1 response balanced with an adequate Th2 component (18). Rats that control infection develop mature granulomas containing eosinophils, whereas rats with an excessive Th1 response develop more inflammatory granulomas with central necrosis and caseation. Early in the course of rat infection, extracellular *C. neoformans* is prominent, but after granuloma formation the percentage of intracellular fungi increases, with a concomitant reduction in fungal burden (45).

Further evidence that macrophages are required for both control and persistence of disease came from the observation that macrophage depletion can prevent yeast dissemination into the mouse brain (46, 47). This result is consistent with the notion that fungal dissemination to the brain involves the transport of viable yeast cells inside host macrophages. The idea that *C. neoformans* has a favorable niche within murine macrophages was directly investigated by constructing a yeast strain that could survive only within acidic environments. During the course of infection, an acidic environment is found solely in the phagosome. This strain, although confined to the phagocytic compartment, was still virulent in natural killer– and T cell–depleted mice, indicating that yeast virulence occurs from the intracellular compartment (47). In the same immunosuppressed mice, depletion of alveolar macrophages delayed mouse death, supporting the concept that the macrophages are a niche for intracellular survival of *C. neoformans* (47).

The Intracellular Life Cycle of *Cryptococcus Neoformans*

Fungal Entry and Recognition

Fungal cell wall components, such as α-glucans, β-glucans, and chitin, are recognized by pattern recognition receptors (PRRs) present in immune cells, triggering cellular activation and, in the case of phagocytic receptors, ingestion of the fungal particle. However, the capsule is highly antiphagocytic, and without opsonins there is no significant ingestion of yeast cells in vitro. Because acapsular *C. neoformans* is readily ingested through complement receptors and/or β-glucan receptors (48), it has been hypothesized that the large polysaccharide capsule conceals most fungal PRR ligands, thereby decreasing phagocytosis by host cells (Figure 2) (49). In fact, for efficient phagocytosis in vitro (Figure 3), opsonization with antibody or complement is necessary, after which phagocytosis proceeds through a complex interplay of Fc receptors, complement receptors (50), and Dectin-1 (51). Despite the capsule's antiphagocytic properties in vitro, *C. neoformans* ingestion occurs readily in vivo. The opsonin or the receptor responsible for in vivo ingestion has not been definitively identified. The complement system is the most likely candidate because complement-deficient animals have greater susceptibility to cryptococcosis (14, 52). *C.*

neoformans spores are acapsular, and thus their surfaces expose more β-glucans than do the surfaces of the yeasts; therefore, when spores are the infectious particles, Dectin-1 and other β-glucan PRRs might be readily activated (8) and mediate rapid ingestion of *C. neoformans*.

Cell wall β-glucans can be recognized by Dectin-1, Toll-like receptor 2 (TLR2), Nodlike receptors, and several scavenger receptors. In addition, CD36 and scavenger receptor F1 (SCARF1) are responsible for immune cell binding of *C. neoformans* in the mouse lung (53). Recognition of the yeast particle is not limited to the immune cell extracellular membrane but continues within the phagolysosome, and even the host cytosol is monitored for the presence of fungal components. In *C. albicans* infection, Dectin-1 and complement receptors accumulated at sites of phagocytosis but dissociated from the phagosome shortly after internalization, while mannose receptors fused into nascent phagosomes, displaying a coordinated cooperation (54). In contrast, in *Aspergillus fumigatus*, Dectin-1 remained within the phagosome and was capable of interacting with β-glucans within the acidic compartment (55). Activation of Dectin-1 by β-glucans in vitro led to enhanced macrophage fungicidal activity, presumably because Dectin-1 mediated inflammasome activation and proinflammatory cytokine production (Figure 4a), which can trigger a more effective antifungal response. Therefore, disguise of β-glucans by the *C. neoformans* capsule may impair maximal macrophage activation. Thus, defects in recognition of *C. neoformans* by Dectin-1 might explain why mice deficient in Dectin-1 do not have increased susceptibility to *C. neoformans* infection (56). This hypothesis has been proven in *C. albicans*, where Dectin-1 dependency is fungal strain dependent due to differences in cell wall composition (57). Other receptors have been shown to be crucial for *C. neoformans* recognition. Both TLR2- (58) and mannose-deficient mice (59) have decreased immunity to cryptococcal challenge, and the TLR9 receptor is important because of cytosolic detection of fungal DNA (60, 61). In summary, mannose receptor, complement receptors, CD36, SCARF1, TLR2, and TLR9 are all crucial receptors for *C. neoformans* recognition in the lung, and cross talk between multiple PRRs is necessary for maximal immune response.

Cells other than immune cells might also recognize the presence of *C. neoformans*, and IL-8 secretion by epithelial cells has been detected (62). Within the lungs, despite extensive adhesion to the epithelium, very little invasion of epithelial cells by *C. neoformans* occurs (63). However, the yeast is commonly found within lung capillaries and can cross the blood-brain and endothelial barriers, which leads to the conclusions that the yeast is able to cross host tissues (64) and that epithelial cells play a role in the pathogenesis of *C. neoformans*.

Phagosome Maturation

C. neoformans has not been shown to interfere with phagosomal maturation. A phagosome containing *C. neoformans* is able to acidify (65), and this acidification is beneficial for fungal replication (65–67). The existing characterization of the *C. neoformans* phagosome shows that lysosomal fusion occurs (Figure 5) and phagosomes quickly acquire an array of phagosomal markers (Figure 6) (65, 66, 68). Autophagic markers colocalize to the *C. neoformans* phagosome (65), but the yeast has not been found within an autophagic compartment. Autophagy mediators may perform functions in this phagosome distinct from their canonical functions; such hypothetical activities would explain why depletion of Atg2,

Atg5, or Atg9 decreases uptake and/or replication of *C. neoformans* (65, 69) and why depletion of Atg5 affects survival after *C. albicans* but not *C. neoformans* challenge.

Evidence for Host Cytotoxicity

Despite normal phagosome maturation, macrophage phagosomes become leaky after *C. neoformans* infection, as measured by light and electron microscopy (70). Leakiness of the phagosome would have a myriad of consequences: loss of acidity, leakage of macrophage-damaging phagosomal enzymes, easy fungal access to cytoplasmic nutrients, and release of strong immunomodulatory capsular components into the cytosol (Figure 4b). At this time, it is not clear whether the leakiness of phagosomes reflects a loss of phagosomal integrity due to macrophage damage, a direct effect of the fungus, or a combination of both. It is also hard to reconcile the fact that the yeast prefers an acidic phagosome with the notion of fungi residency within a leaky, nonacidic phagosome.

Reports of fungal damage to host macrophages are scarce. Lipid peroxidation was observed in rat alveolar macrophages exposed to *C. neoformans*, which presumably occurs as a result of excessive ROS production by the macrophage (71) and not due to direct fungal toxicity. In vivo, cells that have ingested *C. neoformans* display features of affected lysosomes; they are known as hueco cells, after the Spanish word for hole, given their perforated appearance in electron microscopy preparations (43). Capsulated, but not acapsular, *C. neoformans* can trigger apoptosis in macrophages (72), and this observation has been replicated for isolated capsular components (73). Phagocytosis can stimulate proliferation of macrophage cells (74, 75), yet in prolonged *C. neoformans* infection, ingestion of yeast cells specifically inhibited cyclin D1 expression (75) and decreased macrophage mitosis, indicating cell cycle arrest (76). Similarly, the presence of extracellular yeast triggered aneuploidy and cell cycle impairment in macrophages (72). The realization that fungi, like bacteria such as *Mycobacterium tuberculosis* (77), can manipulate the host cell cycle to their advantage is an exciting development in fungal pathogenesis. However, the type of macrophage adaptations necessary to support the observed long-term residence of fungal pathogens has not been elucidated.

Killing of Cryptococcus neoformans

Human macrophages restrict *C. neoformans* growth for up to 24 h after infection (78), a finding indicative of damage to the fungus. Within the phagosome, the yeast is exposed simultaneously to low pH, ROS, reactive nitrogen species, and nutrient starvation (79). These challenges are counteracted by equally powerful mechanisms on the yeast side. Upon ingestion, the yeast upregulates gene expression of oxidative stress enzymes (80), starvation responses, and the autophagic machinery (81). These collaborate with the antioxidant properties of fungal melanin and the capsule to efficiently protect the fungus from host attack. In a model of NADPH oxidase–null mice, cryptococcal infection is contained and the fungal load in both brain and lung is decreased (82), suggesting that inflammatory ROS are prejudicial to the host rather than to the fungus. One antimicrobial molecule proven to be inhibitory to *C. neoformans* in acidic conditions is NO (83). The enzyme that produces NO, iNOS (inducible nitric oxide synthase), is present in *C. neoformans* granulomas in the lung (37, 42, 84), and NO has a protective role in the cryptococcosis mouse model (85, 86). The

understanding of these complex effects is hampered by the difficulty in separating the direct fungicidal and indirect immunoregulatory effects of NO, but because *C. neoformans* with defective nitrosative defenses is only slightly less virulent than is wild-type *C. neoformans* (87), immunoregulation seems to be the predominant effect of NO.

T cells and natural killer cells exert direct antifungal activity, at least in vitro (88, 89), through an unknown mechanism. Neutrophils and dendritic cells can kill opsonized fungi through oxidative and nonoxidative mechanisms (90, 91), and the myeloperoxidase system contributes significantly to antifungal activity against *C. neoformans*, given that myeloperoxidase-knockout mice have dramatically decreased survival after cryptococcal infection (92). Nonoxidative mechanisms include Cathepsin-B-induced structural changes and rupture of the fungal cell wall (93) in dendritic cells, whereas neutrophils have been reported to use both oxidative burst and nonoxidative molecules such as calprotectin and defensins (91).

In macrophages, microbicidal activity depends on macrophage activation, in which Th1-type responses result in the upregulation of ROS, reactive nitrogen species, proteases, and lipid mediators (94), all of which would render macrophages more effective in pathogen killing. Such Th1 stimulation can also decrease phagosomal hydrolase activity to increase major histocompatibility complex presentation and stimulation of adaptive immunity (95). However, in the case of *C. neoformans* infection, even IFN-γ stimulation of macrophages failed to elicit efficient killing in vitro (78). Therefore, the contribution of macrophages' oxidative and nonoxidative defenses to fungal control remains unknown.

Nonlytic Exocytosis

Upon phagocytosis, *C. neoformans* can undergo morphological changes, such as capsular enlargement, that aid its survival within, and even its escape from, host phagocytes (96). Some of these changes include fungal giant cell (titan cell) formation (97, 98), cell-to-cell spread (99), and nonlytic exocytosis (NLE) (100, 101). The presence of mechanisms to flee from phagosomes or traverse to an adjacent cell is compelling evidence of the yeast's adaptation to an intracellular lifestyle. NLE occurs after phagosomal maturation and requires fungal viability (100–102). Curiously, phagosomal permeability always precedes NLE, whereas actin flashes around the phagosome seem to counteract fungal escape (Figure 7) (103). Interference with host cytoskeletal machinery decreases NLE (104), and yeast cells have been found to interact with host cytoskeletal Rac1, a small GTP-binding Rho family protein, to penetrate the blood-brain barrier (105), indicating that the host cytoskeleton can be subverted to promote fungal escape. The most surprising feature of NLE is how little macrophage damage ensues immediately afterward, with the exception of giant vacuole formation in the cytoplasm of the host cell (100).

NLE appears to be tightly modulated by macrophage permissiveness. Macrophages activated by Th2 cytokines in vitro showed an increase in intracellular proliferation and a decrease in extrusion rate when compared with nonstimulated macrophages (106). Th2 cytokines enhance iron uptake and storage by macrophages (107), which may transform the phagosome into a more hospitable environment for the yeast. As mentioned above, acidification of the phagosome is beneficial for *C. neoformans* (65), and blockage of

acidification increases NLE rates (102, 104). These results could be interpreted to suggest the curious hypothesis that a less favorable intracellular niche leads to increased fungal escape via NLE.

Trojan Horse Hypothesis for Extrapulmonary Dissemination

The Trojan horse hypothesis posits that a pathogen gains entry into the blood-brain barrier through dissemination within immune cells (108). In this scenario, the macrophage functions as a Trojan horse, carrying the fungus throughout the body and contributing to dissemination and the breaching of epithelial and endothelial barriers. For *C. neoformans*, a Trojan horse mechanism for dissemination is supported by the observation that depletion of alveolar macrophages prevents brain dissemination (47). Similarly, injection of ex vivo infected macrophages into mice resulted in increased brain fungal burden (46). However, alternative mechanisms of penetration into the brain are possible, such as active penetration of endothelial cells, by either a transcellular or paracellular mechanism (64, 109), and *C. neoformans* proteins that contribute to differential lung/brain infection ratios have been identified in a mutant screen (110, 111). For example, phospholipase B mutants have reduced virulence and invasion of the brain (112). Phospholipase B was found to interact with host cytoskeletal Rac1 to promote brain invasion (105), supporting the idea that *C. neoformans* may use transcellular mechanisms in addition to the Trojan-horse mechanism.

Cryptococcus Neoformans is An Intracellular Pathogen

Establishment of a latent intracellular residency is a very common outcome after phagocyte–fungal cell interactions (see sidebar, The Amoeba-Macrophage Connection). Although *C. neoformans* is not an obligate intracellular pathogen, intracellular residency is an environment where *C. neoformans* can persist and even travel, if we attribute brain invasion to migratory infected macrophages. However, it remains unclear why latency, and not eradication of infection, is such a common outcome. One explanation postulated the damage-response framework (113), which was further developed with the tolerance hypothesis (114). According to the tolerance hypothesis, resistance mechanisms minimize pathogen burden, whereas tolerance mechanisms maximize host function without affecting microbe burden. Consequently, brain, lungs, and heart are the most susceptible organs to immune damage (114). Two of these organs are major targets of *C. neoformans*. In chronic infection models, the yeast spreads to spleen and liver early in infection but is later cleared (42), consistent with a lower risk to these organs of immune damage (due in part to their greater regenerative capacities). Thus, control of infection by intracellular latency, but not clearance, might be a tolerance mechanism to minimize brain and lung damage. Within this postulate, intracellular residency is a tolerance mechanism that would minimize both direct fungal damage to the host and exposure of fungi to the immune response (which would trigger immunopathology), allowing maximal host function (114). These considerations raise the question of why *C. neoformans* has particular tropism for the lung and brain, but not the heart, for which we cannot formulate a credible explanation. When cryptococcal pathogenesis is viewed in the context of the tolerance hypothesis, it appears that fungal intracellular residence is an outcome that presents advantages to both organisms.

Conclusions and Unresolved Questions

There is still much to be discovered regarding the survival of *C. neoformans* within macrophages and its capacity for lung intracellular residence in pathogenesis. Most cases of cryptococcosis are initiated by lung pathology, a finding that provides evidence consistent with a pulmonary reservoir for latency. However, animal studies show that dissemination to the brain occurs shortly after pulmonary infection, which suggests that the brain could also be a reservoir for the yeast. If so, how does the yeast establish latency within the immunoprivileged brain, and are there particular mechanisms of fungal control within the brain?

Within the lung, yeast control is achieved through the formation of specialized granulomas. Granulomas originate from immune cell cooperation, including macrophages and granulomas generated in vitro that have already been used as a *C. albicans* infection model (115). That macrophage granulomas and giant cells possess cellular and molecular characteristics distinct from those of macrophages (116) could explain the observed reduced fungicidal capacity of macrophages in vitro. An alternative explanation could be that immune cells must cooperate, meaning that macrophages would have to acquire a microbicidal molecule from other immune cells.

Microbial ligands can activate innate immunity in the absence of adequate adaptive immunity (117). Our results (51) have shown an increase in fungicidal activity due to β-glucan stimulation. Given that protection could be elicited with proper innate cell stimulation, without the need for $CD4^+$ T cells, we suggest that microbial ligands might have therapeutic value, in particular for immunocompromised patients in whom proper T cell stimulation is not possible.

In conclusion, *C. neoformans* is capable of surviving within mammalian hosts, contained within the intracellular environment of macrophages. The intracellular residency might reflect the most advantageous equilibrium for the host and the pathogen duo and seems to have evolved serendipitously from an ancient relationship with amoebae. Understanding the features of intracellular life can help to prevent *C. neoformans*–associated deaths.

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The Amoeba-Macrophage Connection

Cryptococcus neoformans is a soil organism that has no requirement for mammalian pathogenesis in its life cycle. Why would *C. neoformans* develop such a sophisticated intracellular pathogenic strategy? Studies of the interaction of *C. neoformans* with amoebae suggested how this strategy might have evolved. Amoebae are predators on *C. neoformans* in soil, which can be replicated in a laboratory setting (118, 119). Analysis of the interactions of *C. neoformans* with *Acanthamoeba castellanii* revealed remarkable similarities to the response elicited by interaction with mammalian macrophages; similar virulence factors are required for pathogenesis in both hosts (120). Subsequent studies have established that other phenomena associated with the interaction of macrophages, such as capsule growth and NLE, can be replicated in *C. neoformans*–amoeba interactions (121, 122). On the basis of these observations, the capacity of *C. neoformans* to survive in macrophages and cause disease in mammals was proposed to be the result of selection by such biotic factors as amoebae in the environment (113). According to this synthesis, environmental pressures selected for traits that were needed to survive phagocytic predators and that incidentally also conferred the capacity for mammalian virulence (113).

Future Issues

1. What is the mechanism of control of the primary *C. neoformans* infection?
2. What immune effector mechanism, lost during immunosuppression or loss of $CD4^+$ T cells, is responsible for control of latent *C. neoformans* infection?
3. Are the mechanisms responsible for control of a primary infection the same as those that will control an established disseminated infection?
4. Is it possible to prevent *C. neoformans* from crossing the blood-brain barrier?

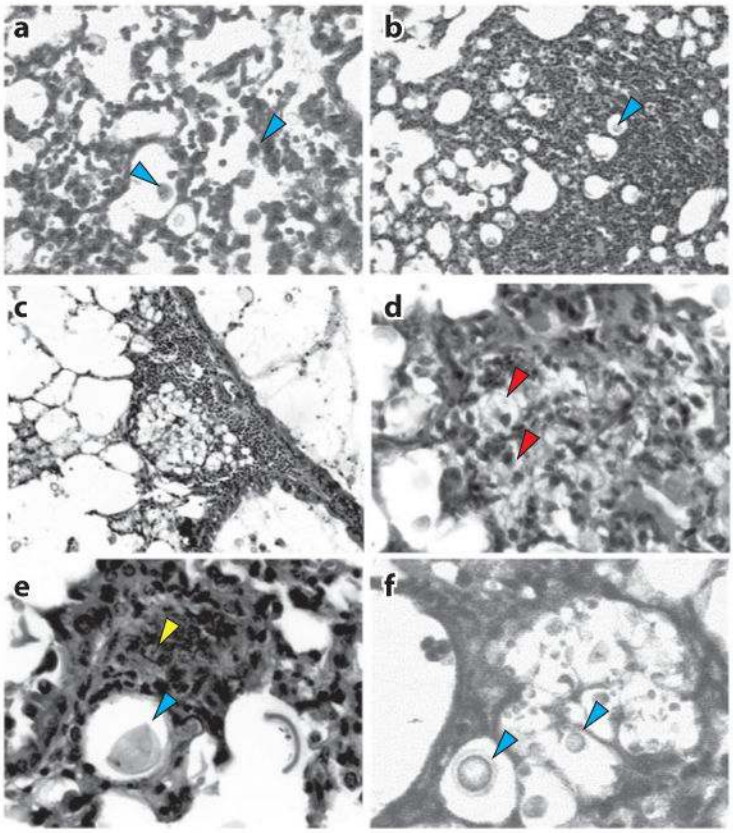

Figure 1.

Histopathology of *Cryptococcus neoformans* lung infection. Photomicrographs of lung tissue from Balb/c mice infected with *C. neoformans* (*blue arrowheads*), stained with hematoxylin and eosin. (*a*) Initial infection, showing diffuse pneumonitis and infiltration of immune cells and yeast into the alveolar space (200×). (*b*) Typical granuloma formation 5 days postinfection (200×). (*c*) Typical granuloma formation 15 days postinfection (100×). (*d*) Magnification of panel *c*, showing the presence of histiocytes (*red arrowheads*) (400×). (*e*) At later stages of infection, giant cells (*yellow arrowhead*) contain *C. neoformans* (400×). (*f*) *C. neoformans* replicating within the alveolar space, visualized by periodic acid–Schiff stain (400×).

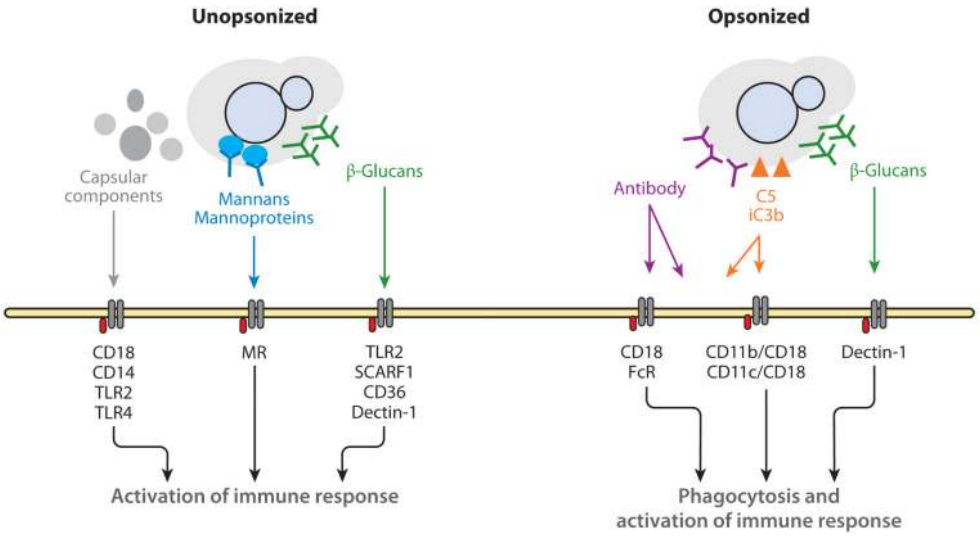

Figure 2.

Schematic of recognition of *Cryptococcus neoformans* by immune cells. Recognition of *C. neoformans* by immune cells depends on several receptors and extensive cross talk between those receptors. Recognition of capsular components was determined in isolation and likely also occurs for the whole capsule. Most of these receptors are not opsonic, meaning they cannot mediate ingestion. The in vivo opsonins are thought to be serum components iC3b and C5, such that the yeast is ingested via cooperation between complement receptors, FcRs, and possibly Dectin-1. Abbreviations: FcR, Fc receptor; MR, mannose receptor; TLR, Toll-like receptor.

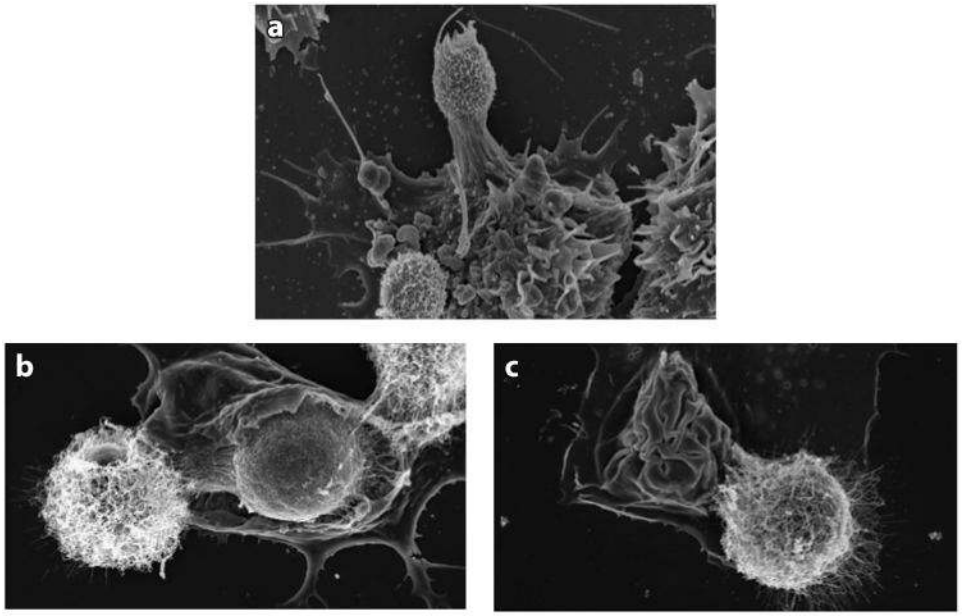

Figure 3.

Scanning electron micrographs showing *Cryptococcus neoformans* and macrophage interaction in vitro. Bone marrow–derived macrophages were infected with antibody-opsonized *C. neoformans*, and macrophage membranes are shown interacting with yeast cells. (*a*) Yeast cells are recognized when macrophage membranes probe the extracellular environment around them. (*b*) Capsulated yeast cells are ingested as the macrophage membrane engulfs them. (*c*) Ingestion is finalized when the membrane closes upon the yeast cell; a neighboring extracellular yeast is also shown. Panel *a* courtesy of Sabriya Stukes; panels *b* and *c* acquired with the help of Julie M. Wolf.

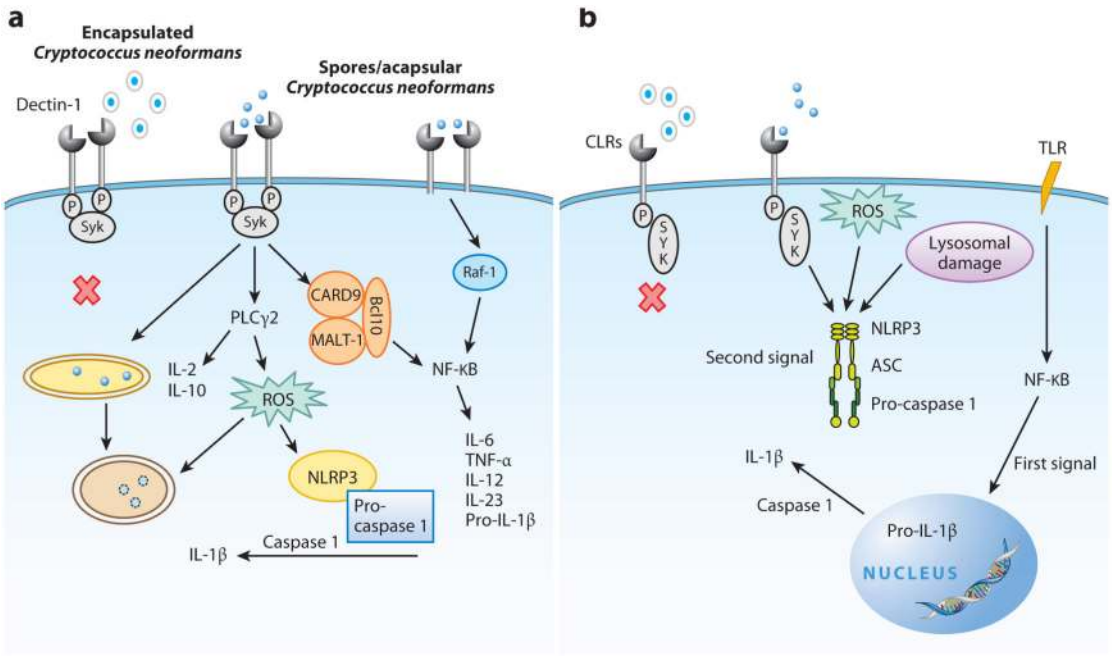

Figure 4.

Schematic of immune signaling cascades triggered by *Cryptococcus neoformans* recognition. (*a*) Dectin-1 signaling pathway. Dectin-1 can induce both Syk-dependent and Raf (Syk-independent) pathways. Dectin-1 can activate macrophages through the Syk pathway, triggering phagocytosis; following phagocytosis, Dectin-1 activation, coupled to ROS production, contributes to inflammasome activation or fungal killing and activates the transcription factor NF-κB through CARD9, triggering inflammatory cytokine production. The Raf-1 (Syk-independent) pathway enhances NF-κB and inflammatory cytokines. (*b*) Inflammasome pathway. The Syk-dependent pathway requires combination of two signals. The first signal, which can be mediated by TLR activation, together with a second signal, such as ROS production and/or lysosomal damage, induces the oligomerization of the NLRP3 complex, activation of caspase 1, and production of IL-1β. Abbreviations: ASC, apoptosis-associated speck-like protein containing a C-terminal CARD; Bcl10, B cell leukemia/lymphoma 10; CARD9, caspase recruitment domain–containing protein 9; CLR, C-type lectin receptor; IL, interleukin; MALT-1, mucosa-associated lymphoid tissue 1; NF-κB, nuclear factor κ-light-chain enhancer of activated B cells; NLRP3, Nod-like receptor family, pyrin domain–containing 3; PLCγ2, phospholipase Cγ2; ROS, reactive oxygen species; Syk, spleen tyrosine kinase; TLR, Toll-like receptor; TNF-α, tumor necrosis factor α

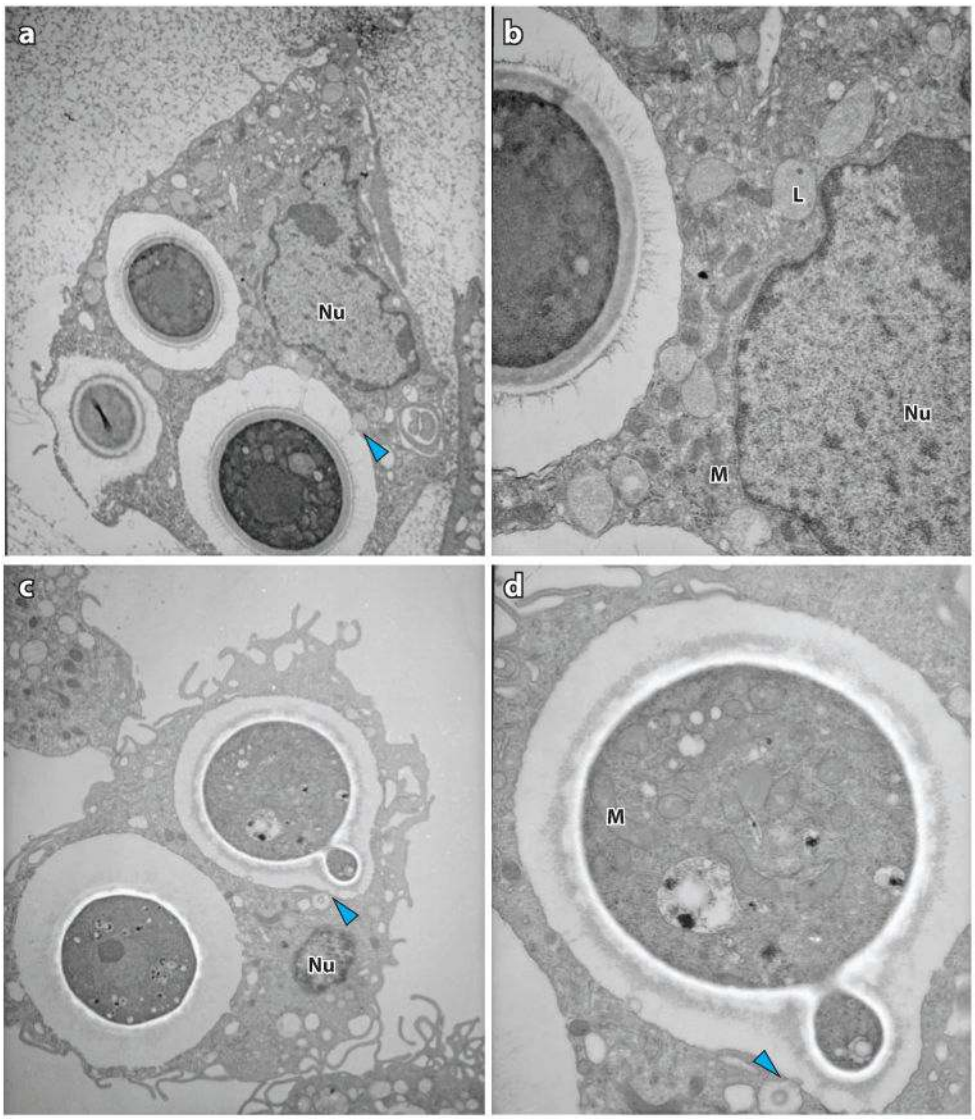

Figure 5.

Transmission electron micrographs showing *Cryptococcus neoformans* and macrophage interaction in vitro. Blue arrowheads indicate possible lysosomal fusion events. (*a*) Macrophage with ingested *C. neoformans*. (*b*) Magnification of panel *a*, highlighting macrophage organelles, particularly lysosomes, in proximity with the phagosome. (*c*) *C. neoformans* budding within a phagosome. (*d*) Magnification of panel *c*, displaying *C. neoformans* organelles. Abbreviations: L, lysosome; M, mitochondrion; Nu, nucleus.

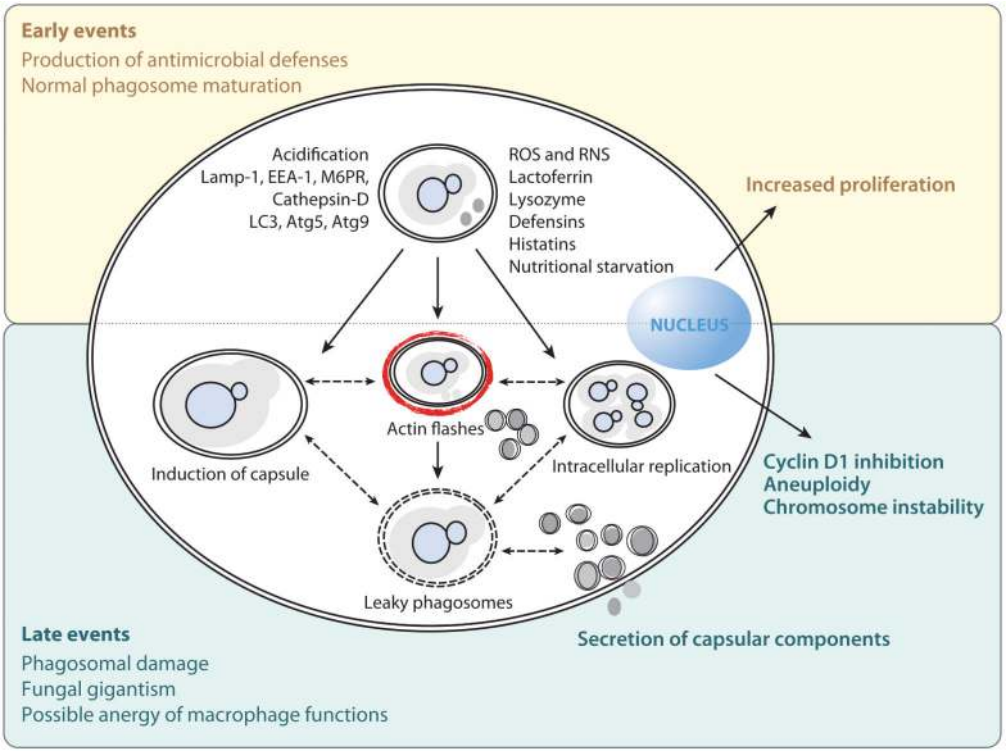

Figure 6.

Phagocytic events upon *Cryptococcus neoformans* ingestion. To date, no manipulation of the phagocytic compartment by *C. neoformans* has been described. The interplay between macrophage fungicidal mechanisms and *C. neoformans* results in host damage, mainly to the phagosomal compartment and to the regulation of the host cell cycle. Abbreviations: ROS, reactive oxygen species; RNS, reactive nitrogen species.

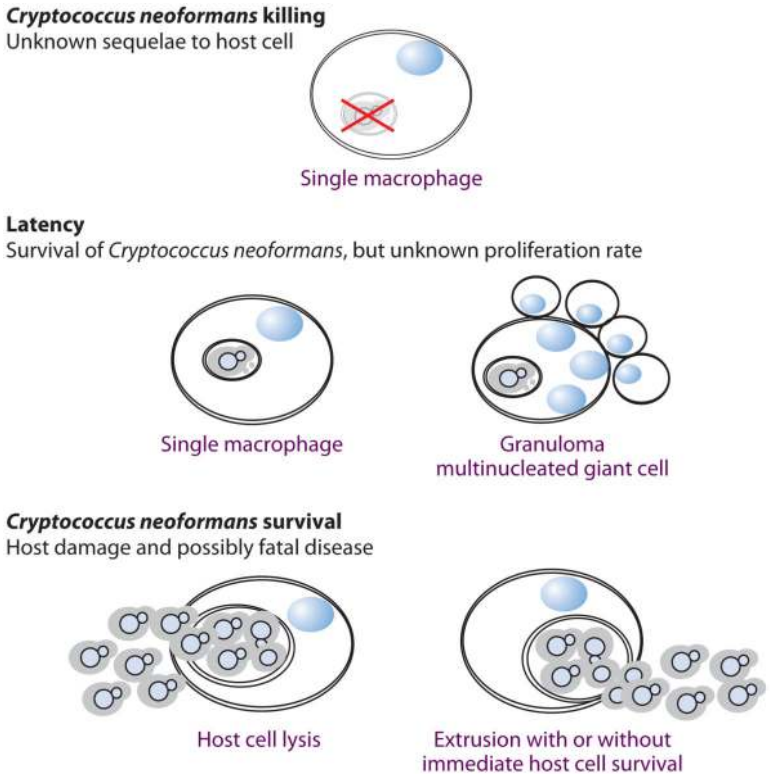

Figure 7.

Possible outcomes for *Cryptococcus neoformans* infection of murine macrophages. The interaction between *C. neoformans* and host macrophages can result in different outcomes, and the frequency with which they occur influences the course of infection.

Table 1

Role of immune components in mouse model of cryptococcosis

Type	Immune polarization	Immune component	Infection route	Outcome when removed[a]	Reference(s)
Recognition/binding	—	CD36	i.v.	Decreased survival	53
		Mannose receptor	i.n.	Decreased survival	59
		C5	i.v.	Decreased survival[b]	14
		C3	i.v.	Decreased survival	52
		TLR2	i.p.	Decreased survival	58
		TLR4	i.p.	No or limited effect	58, 123
		TLR9	i.n.	Decreased survival	61
		Dectin-1	i.v.	No effect	56
Immune cells	—	Neutrophils	i.t.	Increased survival	30
		Eosinophils	i.t., i.n.	Increased survival[c]	29, 31
		Macrophages	i.v.	Decreased survival	26
		Macrophages/dendritic cells	i.t, i.v.	Decreased survival	27
		B cells	i.v.	No effect[d]	124
		$CD4^+$ T cells	i.t.	Decreased survival[c]	11, 125
		$CD8^+$ T cells	i.v.	Decreased survival	11, 126
Cytokines	Th1	IL-12	i.v.	Decreased survival	17
	Th1	IFN-γ	i.v., i.t	Decreased survival	15, 126
	Th1	IL-18	i.n., i.t.	Decreased survival	61, 127
	Th1	TNF-α	i.t.	No effect	23
	Th2	IL-13	i.n.	Increased survival	128
	Th2	IL-4	i.v.	Increased survival	17
	Th1/Th17	IL-23	i.p, i.v	Decreased survival	129
	Th17	IL-17	i.n.	Important for early response	25
	Th2	uPA	i.t.	Decreased survival	130
	Antiinflammatory	IL-10	i.v.	Decreased survival	131
	Inflammatory	IL-6	i.v.	Earlier death	131

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Type	Immune polarization	Immune component	Infection route	Outcome when removed[a]	Reference(s)
	Antiinflammatory	TGF-β	i.n.	Mixed results[c]	132
Effector molecules	—	Nitric oxide synthase	i.t	Decreased survival rate	86
		NADPH oxidase	i.n.	Increased survival[c]	82
		Myeloperoxidase	i.n., i.v.	Decreased survival	92
Signaling molecules	—	MyD88	i.p.	Earlier death	58

Abbreviations: IFN, interferon; IL, interleukin; i.n., intranasal; i.p., intraperitoneal; i.t., intratracheal; i.v., intravenous; TGF, transforming growth factor; TLR, Toll-like receptor; TNF, tumor necrosis factor; uPA, urokinase-type plasminogen activator.

[a] Compared with wild-type mice.

[b] Inferred from correlating mouse strain susceptibility with presence of C5.

[c] No survival study performed, but conclusion is supported by pathology and cytokine profile.

[d] According to Reference 133, B cells play a role in regulating immunity and establishing protection.

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