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Paradoxical roles of alveolar macrophages in the host response to *Cryptococcus neoformans*

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Abstract

Cryptococcus neoformans (Cn) is a fungal pathogen that is a serious health threat to immunocompromised individuals. Upon environmental exposure, infectious fungal propagules are inhaled into the host's lungs. The anticryptococcal actions of alveolar macrophages (AM), the predominant host phagocyte of the innate immune system in the lungs, are fundamental in determining whether containment and clearance of the pathogen occurs by the development of an adapted immune response or whether infection is established and progresses to disease. However, the fungus is also capable of surviving the antimicrobial actions of AM and exploits these host phagocytes to establish infection and exacerbate disease. In addition, there is evidence suggesting that cryptococcosis may occur following reactivation of latent cryptococcal infection. Currently, the role of AM and the fungal factors contributing to latent cryptococcosis are unknown. This review examines the AM-*Cn* interaction and how it affects the development of pulmonary disease with a focus on host and pathogen factors enabling latency to occur.

Keywords

Cryptococcus; cryptococcosis; macrophage; lung disease

WORLDWIDE HEALTH BURDEN OF *CRYPTOCOCCUS NEOFORMANS*

The pathogenic fungus *Cryptococcus neoformans* (*Cn*) is a major cause of morbidity and mortality in immunocompromised individuals. Cryptococcosis is one of the most common opportunistic infections in HIV-infected patients, as it is diagnosed in approximately 1,000,000 individuals/year and is responsible for an average of 600,000 deaths/year **[**1**].** The vast majority of these cases occur in regions of the world where the HIV pandemic persists at alarmingly high rates and access to adequate medical care is limited **[**2**].**

Since the induction of highly active antiretroviral therapy (HAART) as the gold standard treatment in the management of HIV/AIDS, the incidence of cryptococcosis in medically developed countries has significantly decreased **[**3**]**. However, even in countries where HAART has resulted in a decline in opportunistic infections, cryptococcosis remains a serious health concern for non-HIV-infected individuals, such as those with immune

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deficiencies or patients receiving immunosuppression therapy comprised of corticosteroids, monoclonal antibodies or other immunosuppressive agents. In fact, cryptococcosis is the third-leading invasive fungal infection in solid organ transplant recipients and comprises upwards of 60% of all non-HIV cryptococcal disease cases **[**4**].** The number of non-HIV cryptococcosis cases is suspected to increase proportionally with advances in transplantation medicine and immunity manipulation, along with the broadening application of immunosuppressive therapeutic regimens **[**3**]**. Health care facilities have reported mortality rates of 100% within two weeks of clinical presentation when patients are not placed on specific antifungal therapeutic regimens **[**5**].** In addition, acute cryptococcal meningoencephalitis still has a three-month mortality rate of approximately 20% even in patients undergoing advance medical treatment **[**6**].**

Unfortunately, with the possible exception of voriconazole in 2002, no new drugs have been approved in the last 10 years useful in the management of cryptococcosis **[**7**]**. As emphasized in the 2010 Infectious Diseases Society of America (IDSA) guidelines for the management of cryptococcosis, without ideal anti-*Cn* agents available, important principals for the management of cryptococcal infections remain in diagnosing cryptococcosis, regulating host immunity and managing toxicity of anti-fungal therapies, **[**7**].** With the dilemmas of using current drug regimens (i.e. drug efficacy versus patient side effects) as well as unacceptably high mortality rates, the discovery of new therapeutic targets and development of new anti-*Cn* drugs is greatly needed for the treatment and management of cryptococcosis.

CRYPTOCOCCUS NEOFORMANS EXPOSURE AND THE ESTABLISHMENT OF INFECTION

The basidiomycete *Cn* is a free-living environmental detritivore found throughout the world. Environmental isolates have been extracted from soil, pigeon droppings, and decomposing/dead wood from certain tree species **[**8-10**].** Cryptococcal infections in humans are believed to occur following inhalation of desiccated yeast or possibly infectious spores. Upon entering the lungs, as a facultative intracellular pathogen, the infectious *Cn* propagule can reside in the extracellular environment of the alveolar spaces of the lungs or, upon phagocytosis, persist intracellularly within alveolar macrophages (AM). As the predominant resident phagocytes in the lungs, AM possess an essential role in the host immune response to inhaled pathogens. Internalization of *Cn* via receptor-mediated phagocytosis is a pivotal event in the *Cn*-AM interaction because it precedes other AM effector actions, which include the secretion of chemokines and cytokines, recruitment of other immune cells, killing of internalized pathogens, and antigen presentation **[**11-15**].** Following internalization there are three possible outcomes in this host-pathogen interaction: 1) the cryptococcal cells are contained and cleared in a concerted effort of the innate and adaptive immune responses; 2) *Cn* enter a latency stage, allowing for possible reactivation and subsequent symptomatic disease at a later time; or 3) *Cn* survive and the infection progresses to establish pulmonary cryptococcosis, which can disseminate to cause systematic disease including life-threatening meningoencephalitis. Which of these outcomes occurs is dependent on the balance between *Cn* fitness and AM anticryptococcal activity (Figure 1).

ALVEOLAR MACROPHAGES AND THE HOST IMMUNE RESPONSE TO *CRYPTOCOCCUS NEOFORMANS*

AM have an instrumental role linking innate immunity and adaptive immune responses in the lung. Upon interaction with a pathogen, macrophages secrete cytokines that modulate the development and expression of antigen-experienced $CD4^+$ into Th1, Th2, or Th17 effector cells. In turn, specific cytokine profiles produced by these effector $CD4^+$ Th cells augment macrophage function, which determines their capacity to kill internalized pathogens. A polarization of the host immune response towards $CD4^+$ Th1 immunity is well established as an essential element leading to successful clearance of *Cn* in the lungs **[**16-24**]**. Healthy humans mount predominantly a Th1-mediated response to inhaled *Cn* cells, resulting in the formation of a granuloma encapsulating the infection site that acts as a physical barrier, which prevents access to vasculature and egress from the lungs to extrapulmonary sites **[**25, 26**].** Th1-associated cytokines, specifically tumor necrosis factor alpha (TNFα) and interferon gamma (IFNγ), evoke classic activation of macrophages, which upregulates NOS2 enzyme to produce fungicidal NO from L-arginine, which kills internalized *Cn* **[**27**]**. The importance of the Th17 immune response to *Cn* in the host is still being defined, but initial studies suggest it may positively contribute to a host's ability to prevent cryptococcosis **[**24, 28-30**].** Research into the effect of the Th17-associated cytokines on the *Cn*-macrophage interactions has shown Il-17 to activate macrophages and limit intracellular proliferation of *Cn* cells **[**31**].** In contrast, induction of a Th2 response is non-protective to the host, as demonstrated in mouse models and analysis of clinical cases **[**30, 32, 33**].** Th2-associated cytokines, such as IL-4 and IL-13, induce alternative activation of macrophages, which increases phagocytosis and intracellular growth of *Cn* **[**27**]**. Therefore, the fate of *Cn* cells internalized by AM, and consequently the outcome of the host, is greatly influenced by the CD4+ Th response that is evoked. This has been shown in mouse and rat models of pulmonary cryptococcosis, where differences in susceptibility to cryptococcosis between species were found to correspond to the efficacy of anticryptococcal activities of AM **[**34**]**. However, it should also be noted that, as demonstrated by Zhang *et al* using IL-4/IL-13 knockout mouse model, a robust Th1/Th17 immune response is not sufficient to prevent disease from highly pathogenic *Cn* strains **[**30**].** Thus, future studies are essential to elucidating host factors affecting susceptibility to cryptococcosis.

As alluded to above, phagocytosis is necessary for containment and clearance of pathogenic microorganisms from the lungs but internalization can also be detrimental and injurious to the host, depending on whether AM are classically activated (Th1-/Th17-associated cytokines) or alternatively activated (Th2-associated cytokines). Unlike many intracellular pathogens, *Cn* does not actively avoid the potentially microbicidal phagolysosome but, in fact, can readily survive and proliferate within its confinements **[**35-38**].** Research suggests macrophages may serve as a protective microenvironmental niche enabling *Cn* to avoid the fungicidal actions of other immune cells. *Cn* cells contained within host phagocytes are capable of egress from the lungs and dissemination to the central nervous system, where they can cause life-threatening meningoencephalitis **[**39-42**].** This dichotomy in the role of phagocytosis in host susceptibility to microbial infection is exemplified in the pathogenesis of *Cn* where, under conditions when phagocytes are unable to kill internalized *Cn*, such as

during immunosuppressive states, intracellular residency may be beneficial to the fungal cells by enhancing growth and providing refuge **[**34, 41-43**].** Currently, factors influencing whether AM facilitate anticryptococcal actions or act as a protective environmental niche are not fully defined.

ALVEOLAR MACROPHAGES IN LATENT CRYPTOCOCCOSIS

Since *Cn* causes disease primarily in cases of immune deficiency, one would presuppose that exposure occurs after the development of an immunocompromised condition. However, intriguingly, research suggests latent *Cn* infection may precede the immunodeficient condition and its reactivation may result in clinical cryptococcosis cases. For example, epidemiology studies of cryptococcal cases from HIV+ immigrant patients showed that the infectious strain genotypes were from the patients' countries of origin, acquired years before immunosuppression [44, 45]. In addition, seroprevalence studies have shown the majority of healthy children to possess antibodies reactive to the cryptococcal capsule component glucuronoxylomannan (GXM), suggesting *Cn* exposure occurs during childhood **[**46-50**].** These studies suggest that, for many infections, initial *Cn* exposure occurs early in life, often during childhood, followed by symptomatic disease during the acquisition of an immunocompromised state.

A model of pathogenesis, where *Cn* exposure leads to latent infection and, if immune suppression occurs, reactivation of dormant *Cn* cells results in symptomatic disease, agrees with the concept of the damage-response framework of microbial pathogenesis presented by Casadevall & Pirofski. In this proposition, host damage serves as the essential factor in the host-pathogen interaction facilitating disease by a normally non-pathogenic organism **[**51**]**. Recent research has shown immunosuppression of rats previously challenged with *Cn* induces reactivation of the latent infection, thus mirroring the *Cn* pathogenesis believed to occur in clinical cases **[**51**]**. Yet while conditions affecting cell-mediated immunity could obviously serve as the injurious event to allow reactivation, the host factors facilitating *Cn* latency in an individual with a seemingly intact immune system are unknown.

Since *Cn* can survive and proliferate in macrophages, it is intriguing to wonder whether *Cn* localization (i.e. extracellular versus intracellular within AM) influences its pathogenesis and possible latency. Animal models of pulmonary cryptococcosis have shown that localization of cryptococcal cells in the lungs is not static but, instead, is dynamic over the course of infection and disease progression. Analysis of the lungs of C57BL/6 mice infected with the ATCC *Cn* strain 24067 revealed that approximately 40% of cryptococcal cells were intracellular 2 hours post infection and the percentage of intracellular *Cn* peaked 8 hours post-infection (approximately 85%). A dramatic shift towards extracellular localization occurred after 24 hours, with only about 15% of cryptococcal cells observed within host phagocytes at this later time period. Intriguingly, beginning with day 7 after infection and continuing through day 28, intracellular *Cn* were predominantly observed **[**52**]**. It has been shown that *Cn* strains with mutations affecting their preferential localization within either extracellular spaces or host phagocytes modulate their virulence in murine pulmonary cryptococcosis models **[**41, 53**].**

Immunocompetent Fisher rats, whose host immune response and the resulting *Cn* pathogenesis more closely resemble pulmonary infections in immunocompetent humans than that of mice **[**54**]**, control and contain the ATCC *Cn* strain 24067 with granulomatous inflammation without clinical symptoms **[**36**].** Histopathological analysis of the lungs of immunocompetent Fisher rats infected using a pulmonary cryptococcosis model show *Cn* to have a great propensity for intracellular localization at all time points examined, beginning at 1.5 months post-infection through 18 months post-infection, thereby suggesting that host phagocytes assist in the establishment of latent cryptococcosis. In contrast, the lungs of rats treated with an immunosuppressive regimen of dexamethasone have severely decreased inflammation and granuloma formation, resulting in significantly higher lung fungal burdens and substantially increased extrapulmonary involvement, in comparison to immunocompetent rats. Interestingly, in lungs from dexamethasone-treated rats, the majority of cryptococcal cells are located within the extracellular environment 1.5 months post-infection but are distributed equally between intracellular and extracellular locations 12.5 months after infection **[**36**].** Together, these animal models of pulmonary cryptococcosis demonstrate that *Cn* localization (extracellular versus intracellular) can be dynamic throughout the course of infection and responsive to the status of the host immune response, thereby affecting the outcome of infection.

The complex host-pathogen interaction between marophages and *Cn* is further exemplified by studies demonstrating how internalized *Cn* actively escape from macrophages through a non-lytic mechanism termed expulsion or phagosome extrusion **[**24, 55, 56**]**. Voelz and colleagues determined systematically that the frequency of expulsion increases with macrophage-activating Th1 and Th17 cytokines, while the frequency of expulsion is diminished with Th2 cytokines, which inhibits the anti-cryptococcal function of macrophages **[**24**].** Macrophages containing intracellular *Cn* employ a novel actin-dependent process to preclude expulsion **[**57**].** Although the rate of this mechanism of non-lytic egress from macrophages is observed at relatively low rates *in vitro*, its occurrence *in vivo* represents a unique event that may influence *Cn* pathogenesis by modulating the AM-*Cn* interaction and possibly affecting reactivation of latent *Cn* cells. It is possible that when an immunosuppressive state is induced, such as during advanced HIV infection when Th1 cells are severely damaged, macrophages containing intracellular *Cn* are no longer capable of keeping the fungal cells internalized and/or in a dormant state, at which time *Cn* cells could employ expulsion **[**27**].** Future research, particularly involving animal models and observations of clinical cases, are required to delineate the role of expulsion or phagosome extrusion in the AM-*Cn* interaction.

CRYPTOCOCCUS NEOFORMANS VIRULENCE FACTORS AND MACROPHAGES

Cn possesses a unique array of virulence factors enabling survival in the lung environment, both as an extracellular and intracellular pathogen, and for the establishment of pulmonary infection. Unlike other intracellular pathogens such as *Mycobacterium* species, *Histoplasma capsulatum*, *Legionella pneumophila* and *Toxoplasma gondii*, *Cn* does not actively avoid phagolysosome acidification **[**38**].** In fact, *in vitro* assays using primary and macrophage-like

cells lines shows *Cn* proliferates better in an acidic environment than under alkaline conditions **[**35, 38, 58**].** Thus, as a free-living organism, Cn has evolved mechanisms to survive in its natural environmental niche that translate to the intracellular microenvironment of mammalian phagocytes.

Cn has three preeminent virulence factors: a polysaccharide capsule, laccase activity/ melanin production, and the ability to grow at 37°C. These main virulence traits are pertinent to *Cn* survival in the mammalian host at different stages of infection and are particularly crucial for *Cn* to subsist as an intracellular pathogen following internalization by host phagocytes. The *Cn* capsule has been observed to undergo alterations in both murine models of pulmonary cryptococcosis and clinical histology showing that it is a dynamic entity whose composition and size changes according to various stimuli within its microenvironment in the host **[**44, 59, 60**].** Following phagocytosis, the polysaccharide capsule enlarges and, thereby, imparts greater resistance to microbicidal agents within the lumen of the phagolysosome such as reactive oxygen species (ROS), and antimicrobial peptides **[**61**].** Laccase activity, specifically Lac1, protects intracellular *Cn* from the harsh microenvironment within the host phagocyte as well. Lac1 is a cell wall-associated enzyme that oxidizes exogenous iron and catecholamines to produce the pigment melanin, a molecule linked to antioxidative properties in macrophages **[**62**].** While in the phagolysosome, the iron oxidase activity of Lac1 protects internalized *Cn* by competing with AM for phagosomal iron and preventing the formation of Fe (III), thus inhibiting macrophage ability to produce hydroxyl radicals **[**62-64**].**

Other intracellular pathogens, such as pathogenic *Mycobacterium* species **[**65, 66**]** and *Francisella tularensis* **[**67**],** also modulate host iron homeostasis, thereby lessening macrophage antimicrobial actions. Laccase also produces the immunosuppressing prostaglandin, PGE2, which reduces the ability to recruit and activate macrophages [68-70]. The ability of *Cn* to tolerate the high body temperature of the mammalian host is connected to the gene product of *CNA1*, calcineurin A, which is a Ca^{2+}-calmodulin-regulated protein phosphatase [71]. In addition to modulating temperature sensitivity, calcineurin A-linked signaling pathways control induction of genes required to survive under metabolic and oxidative stress, such as those characterizing various environments of the host, including that within host phagocytes **[**72**].**

CRYPTOCOCCUS NEOFORMANS INTRACELLULAR SURVIVAL FACTORS AND MACROPHAGES

The capacity of a pathogen to survive and propagate in the host is often thought of as its pathogenic fitness [73]. As cryptococcosis may occur following latency and reactivation, a key to understanding *Cn* virulence and pathogenesis may be to elucidate genes and pathways providing cryptococcal cells the pathogenic fitness to subsist for extended periods of time within the host. Fan and colleagues provided an important look into *Cn* factors affecting intracellular survival by examining the transcriptional response of *Cn* following phagocytosis by macrophages. In this work, transcription profiles of the clinical isolate the H99 *Cn* strain were examined at 2 hours and 24 hours post-internalization by the murine

macrophage-like J774A.1 cells. After deducing the magnitude of change in phagocytic-specific gene expression and using a threshold of two-fold or greater compared to internal controls, there were 157 down-regulated genes and 123 up-regulated genes. Importantly, the gene expression profile of internalized *Cn* differed at 2 hours post-internalization compared to 24 hours post-internalization **[**74**]**. Several of these genes, which bestow the ability to tolerate harsh conditions during the intracellular parasitism of *Cn*, have garnered significant attention. Of particular interest are the factors contributing to the aptitude of *Cn* cells to persist in the phagolysosome, as host phagocytes may serve as a protective microenvironment niche under specific conditions (Figure 1).

Several *Cn* genes and pathways that affect intracellular survival independent of the three classical virulence factors have been identified. *Cryptococcus* species express superoxide dismutases 1 (SOD1) and −2 (SOD2) that produce hydrogen peroxide and molecular oxygen from antimicrobial molecule superoxide released by phagocytes **[**75**].** Both SOD1 and SOD2 protect from intracellular antimicrobial actions, albeit by different roles as they differ in their biochemical requirements and cellular localization **[**75-77**].** Through a Pma1-dependent mechanism(s), inositol phosphosphingolipid-phospholipase C1 (Isc1) imparts defense from acidic, oxidative, and nitrosative stresses of the phagolysosome **[**53, 78**].** Isc1 is an enzyme that metabolizes fungal inositol sphingolipids into phytoceramide, and is essential for intracellular survival by imparting defense from acidic, oxidative, and nitrosative stresses of the phagolysosome **[**53, 78**].** In murine models of pulmonary cryptococcosis, the Δ*isc1* mutant strain is almost exclusively localized in the extracellular environment of the lung. Phospholipase B1 (Plb1) is a cell membrane-associated enzyme that can be cleaved from its glycosylphosphatidylinositol (GPI) anchor and secreted from the cryptococcal cell **[**79, 80**].** Plb1 has several enzymatic activities: it can function as a PLB, a lysophospholipase (LPL), and a lysophospholipase transacylase (LPTA) to cleave host lipids. The Δ*plb1* mutant strain has an intracellular growth defect attributed to the production of fungal eicosanoids, which are capable of scavenging macrophage-derived arachidonic acid **[**81**].** *Cn* metabolism of host arachidonic acid diminishes the host cellular pool, which is required for critical macrophage functions, while producing fungal eicosanoids that may protect *Cn* from the host response.

The dynamic nature of *Cn* as an intracellular pathogen responding to a host microenvironment has also been shown when examining membrane-associated transporters regulating nutrient uptake from the external environment, such as carbohydrate transporters and nitrogen starvation-associated transporters located on the plasma membrane. The expression levels of the ferro-O_2-oxidoreductase Fet3 and the iron permease Ftr1, both of which are molecular components involved in iron transportation across the plasma membrane, were significantly upregulated over time during intracellular residency **[**74**]**, showing that sequestering metal molecules from the host is an aspect of nutritional acquisition important to gene regulatory networks of *Cn*. The iron-responsive transcription factor Cir1 has been identified as a regulator of capsule formation, melanin formation in the cell wall, and temperature sensitivity through its control of genes modulating iron acquisition **[**82**].** In addition, the ferroxidase Cfo1, which is essential for the reductive uptake system that acquires iron from host transferrin during infection, is upregulated when *Cn* resides in an iron-limited environment **[**83**].**

Similarly, the acquisition of copper is also vital to *Cn* to survival within the host. The cryptococcal copper dependent transcription factor 1 (Cuf1) is required for growth and virulence factor expression in the presence of low copper concentrations, such as the phagolysosome **[**84**].** The importance of copper on the intracellular parasitism of *Cn* is further exemplified by the fact that the Cuf1-dependent copper transporter, Ctr4, is highly expressed during intracellular residence **[**84**].** Mutant strains with impaired ability to acquire both iron and copper from the host have either attenuated virulence or are avirulent, and clinical strains having intrinsically reduced *CTR4* expression were less likely to cause meningitis rather than more localized pulmonary disease in a cohort of organ transplant patients **[**83, 84**].** These transcription factors, which respond to metal molecules in the host environment, make intriguing therapeutic targets to complement the antifungal drugs amphotericin and fluconazole **[**83**]**.

Research has found internalization by macrophages induces autophagy in *Cn* **[**74, 85**].** Autophagy is a pro-survival adaption of eukaryotes initiated in response to both extracellular stresses, such as nutritional deprivation, and intracellular stresses, including damaged proteins and organelles. To survive within a host phagocyte, internalized microbial pathogens must scavenge nutrients from the vesicles of the phagocytic pathway in which they reside and undergo necessary metabolic alterations. As carbon sources and micronutrients are limited in pathogen-containing vesicles, induction of autophagy provides a mechanism to prolong intracellular survival by recycling organelles and cytoplasmic proteins to provide essential elements for survival. *Cn* autophagy gene *ATG3* is induced at 2 hours after phagocytosis while *ATG9* is induced after 24 hours of internalization within murine macrophage-like J774A.1 cells **[**74**].** Autophagy has been demonstrated directly to occur in *Cn* following phagocytosis by J774A.1 cells through microscopic analysis of the localization of Atg8, a microtubule-associated protein that is induced and associated with vesicles during autophagy **[**85**].** A *Cn* mutant strain lacking the Vps34 phosphatidylinositol 3-kinase (*vps34*Δ), which is known to be involved in autophagy in ascomycete yeast, was defective in the formation of Atg8-labeled vesicles and was rapidly killed within hours by macrophages following phagocytosis *in vitro*, despite normal growth in fungal media at 37°C **[**85**].** Importantly, *vps34*Δ is avirulent in a murine model pulmonary cryptococcosis, where the cryptococcal cells are amazingly killed in the lungs within hours of challenge **[**85**].**

CONCLUSIONS

AM serve as a vital link between innate immunity and the development of an adaptive immune response. The AM-*Cn* interaction is part of an intricate host-pathogen relationship that greatly influences whether cryptococcal cells are contained and cleared from the lungs or whether cryptococcal cells persist to establish infection and disease. Under conditions not fully defined, *Cn* can survive within AM to seemingly exploit the intracellular residency within these host phagocytes. The fact that cryptococcal cells adapt to survive and persist in the host for extended periods of time supports the view that the ability to establish infection, whether it leads to acute or latent disease, has a strong component of pathogenic fitness.

Therefore, future therapeutic regimens should take into account *Cn* pathogenic fitness as it relates to the status of the host immune system. Research also suggests reactivation of latent cryptococcal cells residing within lungs may cause a significant proportion of cryptococcosis cases. Therefore, to identify factors modulating the pathogenic fitness of *Cn*, development of a consensus animal model of latent pulmonary cryptococcosis is required and greater explorations into clinical cases are necessary. Lastly, the *Cn* factors enabling the fungus to survive for an extended period of time and/or reactivate within the host make intriguing targets for drug development. For example, in the case of organ transplantation, drugs can be administered prior to manipulation of host immunity to preclude reactivation and prevent cryptococcosis. The advancements in knowledge thus obtained from these future studies defining host factors, particularly involving macrophages, and distinguishing *Cn* factors contributing to latent infection and reactivation hold a great degree of potential to prevent cryptococcosis in high-risk patient groups, such as HIV-infected individuals and patients beginning immunosuppressive therapy.

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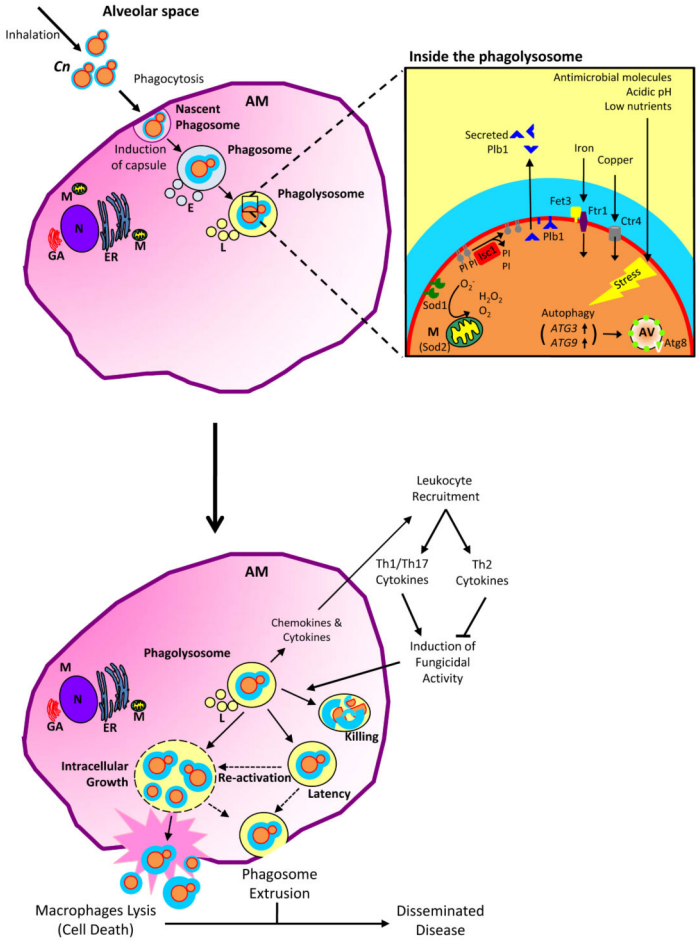

Figure 1.

Strategies for *Cryptococcus neoformans* survival in host alveolar macrophages. Following inhalation, infectious *Cryptococcus neoformans* (*Cn*) propagules enter the alveolar spaces of the lungs and are confronted by resident alveolar macrophages (AM). Upon phagocytosis, the nascent phagosome surrounds the internalized cryptococcal cell. Phagosome maturation proceeds with the fission and fusion of vesicles including endosomes (E) and then lysosomes (L), which ultimately result in formation of the phagolysosome. Antimicrobial molecules, acidic pH, and low level of nutrients all normally contribute to the killing of internalized pathogens. In addition to its polysaccharide capsule, laccase activity/melanin production, and ability to grow at 37°C, *Cn* has several other mechanisms enabling it to survive in the phagolysosome. *Cn* expresses superoxide dismutases 1 (SOD1) and −2 (SOD2) that catalyze the dismutation of antimicrobial superoxide (O_2^-) to produce hydrogen peroxide (H_20_2) and molecular oxygen (O_2). Inositol phosphosphingolipid-phospholipase C1 (Isc1) metabolizes fungal inositol sphingolipids into phytoceramide and is essential for intracellular survival by imparting defense from acidic, oxidative, and nitrosative stresses. Phospholipase B1 (Plb1) is associated with the cell membrane but can also be cleaved from its glycosylphosphatidylinositol anchor and secreted. Plb1 can act as a PLB, a lysopholipase (LPL), and a lysophospholipase transacylase (LPTA) to cleave host lipids. Plb1 contributes to the production of fungal eicosanoids, which are capable of

scavenging macrophage-derived arachidonic acid. Ferro-O_2-oxidoreductase Fet3 and the iron permease Ftr1, molecular components involved in iron transportation across the plasma membrane, and the Cuf1-dependent copper transporter, Ctr4, are highly expressed during intracellular residency in order to sequester the metal molecules indispensible for survival. *Cn* autophagy genes *ATG3* and *ATG9* are stress-induced, resulting in the formation of autophagic vesicles (AV) possessing Atg8. There are three possible outcomes of the AM-*Cn* interaction: 1) the cryptococcal cells are contained and cleared in a concerted effort of the innate and adaptive immune responses; 2) *Cn* enter a latency stage, allowing for possible reactivation and subsequent symptomatic disease at a later time; or 3) *Cn* survive and the infection progresses to establish pulmonary cryptococcosis, which can disseminate to cause systematic disease including life-threatening meningoencephalitis. Which of these outcomes occurs is dependent on the balance between *Cn* fitness and AM anticryptococcal activity.