Microscopic fungi comprise a large number of microorganisms of very different characteristics. Among them, a few can cause disease in humans, especially among immunocompromised patients [1]. Opportunistic invasive fungal diseases have become a problem of concern for the national health systems worldwide due to their high associated mortality and economical cost [2,3].

The main human fungal pathogens belong to the genera Candida, Aspergillus, and Cryptococcus. All of them are cause disease in immunosuppressed patients, so they are considered opportunistic pathogens. The mechanisms involved in invasive fungal disease are different and involved both fungal and host elements. Some of pathogenic mechanisms are shared by all the fungal pathogens, such as the ability to grow at physiological temperature, resistance to the immune challenges (such as free radicals), and others. But the case of Cryptococcus offers an excellent model to investigate fungal pathogenesis for several reasons. First, it is a pathogen of concern because it has a high prevalence in certain geographical regions. But in addition, this yeast has developed some pathogenic mechanisms and acquired some virulence traits that make it an unique fungal pathogen. In this review, I will summarize the main reasons why Cryptococcus is a different fungal pathogen.

**Epidemiology, incidence and disease**

Cryptococcus spp are basidiomycetes yeasts, which is a different characteristic from most human pathogenic fungi, which are ascomycetes. Its metabolism is mainly respiratory, so its growth is highly dependent on the presence of oxygen [4]. From a structural point of view, Cryptococcus spp present a unique phenotypic trait, which is the presence of a capsule that surrounds the cell body.

Cryptococcus spp are widely found in the environment, but there are two species that have been associated to disease in humans: Cryptococcus neoformans and Cryptococcus gattii [5]. These two yeasts are very closely related, but present differences in the epidemiology and disease in humans. The most prevalent is C. neoformans, which behaves as opportunistic pathogen in immunosuppressed patients. Cryptococcus gattii, in contrast, has a lower incidence, but can cause disease in immunocompetent patients [5]. Most studies about virulence have focused on *Cryptococcus neoformans,*
and for this reason, this review will focus mainly on this yeast.

Cryptococcus neoformans has been known as causative agent of disease since the 19th century [5]. However, its incidence raised significantly at the end of the 20th century, associated with the emergence of HIV infection, and it was estimated that it could affect around 10% of AIDS patients. The introduction of the antiretroviral therapy has controlled the incidence of cryptococcosis in developed countries, but it is still a major concern in developing areas. Globally, it is estimated that it causes around 180,000 deaths in Subsaharian Africa, although it has also an important incidence in Asia and South America [6,7].

The infection is acquired by inhalation of spores or desiccated conidia from the environment, and the pathogen first colonizes the lung [4]. The immune response of this organ is very specific because it is continuously exposed to exogenous particles [8], and in fact, it is very effective at controlling the dissemination of the cryptococcal cells. However, in immunosuppressed patients, in particular those defective in CD4 T cells, C. neoformans can replicate and disseminate through the organism. The most characteristic clinical outcome appears when it reaches the brain, where it causes meningoencephalitis. This disease is very serious and has a high mortality associated (around 20–50% of affected patients).

Cryptococcus as environmental pathogen

When compared to other fungal pathogens, C. neoformans is very characteristic in the infection route and in the disease caused. For examples, other yeasts, such as Candida spp, are rarely acquired by inhalation and C. neoformans resembles more the infection caused by filamentous fungi or primary fungal pathogens, such as Histoplasma capsulatum. Cryptococcus neoformans has developed some virulence mechanisms that allow the survival in the lung and dissemination to the brain. Interestingly, some of them are also used to infect and cause disease in environmental host [9]. This yeast has a worldwide distribution and it can be disseminated using some birds as carriers [10]. It is believed that the continuous exposure to environmental stress, such as temperature fluctuations and dehydration has selected cryptococcal strains with a higher fitness in mammalian individuals. Furthermore, it has the ability to infect a large number or organisms, such as amoebas, flies, nematodes, Lepidoptera, and even plants as Arabidopsis thaliana [11–15]. Even in the case of mammals, there are reports of infections in a wide range of animals, such as koalas, dolphins, and cats [16,17]. These multiples interactions are believed to be important for the virulence of C. neoformans, because the mechanisms that allow the fungal survival after interaction with these different hosts have selected multiple traits that can be used to adapt and cause disease in humans. Maybe the phenomenon that is best characterized is its ability to survive after the interaction with environmental predators, such as amoeba [18,19]. This cryptococcal ability is very similar to the behavior during the interaction with mammalian phagocytic cells.

Cryptococcal pathogenesis

Infection by C. neoformans is believed to occur through inhalation infective particles. In this sense, it has been shown that cryptococcal spores can germinate and cause disease [20–22]. After inhalation, C. neoformans must survive in the lung and evade the immune response of this organ, which has a specialized immune response [8]. The alveoli are covered by a layer of alveolar macrophages, which are in charge of the phagocytosis and elimination of these challenges. In addition, lymphocytes (such as CD4, CD8) and other myeloid cells (dendritic cells) are also recruited in this organ. The lung also contains the surfactant, which main function is to maintain surface tension of the pleura during the respiration process. In addition, some proteins from the surfactant have antimicrobial properties.

In this complex environment, cryptococcal virulence is dependent on three main types of processes: Adaptation to the host environment, mechanisms of immune evasion, and production of true virulence factors. Although these three processes should be applied to most fungal pathogens, C. neoformans is unique because it has developed many different types for adapting to the host, evade the immune response, and produce true virulence factors [23]. In the next sections, we will provide a brief overview on the main process that make C. neoformans a unique fungal pathogen.

Adaptation mechanisms to host conditions

As any other microbial pathogen, survival in the host involves the induction of adaptation mechanisms to physiological temperature, different sources of nutrients, pH, and oxidative stress (see seminal review in Ref. [24]). For adaptation to these conditions, C. neoformans induces multiple metabolic rearrangements and activation of signaling pathways (such as MAPKs [25]). However, some aspects of adaptation are of great interest. One is the mechanisms that allow growth at our physiological temperature. Environmental fungi normally do not tolerate high temperatures, including the one from our body [26].
And in fact, most pathogenic fungi are those able to replicate at 37°C. This indicates that this adaptation is most probably one of the main factors to become a pathogen in an immunosuppressed host [27]. Some of the processes required for growth at higher temperatures involve antioxidant responses, trehalose accumulation, and activation of the different signaling pathways [28–31].

Another adaptation process required for survival in vivo are those needed for the uptake of some limiting, but essential nutrients such as metals that are required for many metabolic reactions [32]. Among them, the role of iron in cryptococcal virulence is one of the most studied [33]. This metal is required for the proper activity of many enzymes in the cell, and both yeast and host cells have developed mechanisms to efficiently compete and obtain it from tissues and body fluids. In the case of C. neoformans, several proteins are required for efficient iron uptake, such as iron permeases, ferroxidases, and the glycoprotein Cig1, which is chelator of heme groups [34–36].

The pH is another factor can change, not only in the host, but also in the environment, and for this reason, the ability to adapt to different pHs is also required to survive during infection [37]. One of the main proteins required for adaptation to neutral pH is Rim101, which encodes a transcription factor that responds to alkaline pH [38]. Interestingly, this protein also regulates other important features required for virulence, such as cell wall integrity and capsule production [39–41].

Another process required for survival during infection involves the adaptation to free radicals. One of the main mechanisms elicited by the immune response to induce pathogen killing is oxidative and nitrosative stress. In C. neoformans, adaptation to reactive oxygen species depends mainly based on glutathione and thioredoxin [42–44] and mannitol [45]. Regarding resistance to reactive nitrogen species (RNS), several proteins have been involved in this process. Resistance to nitrosative stress depends on the reductive power (production of NADPH) of the cells and it has been shown that isocitrate dehydrogenase is important for this process. This enzyme catalyzes the conversion of isocitrate to α-ketoglutarate, a reaction that also produces NADPH. Absence of isocitrate dehydrogenase results in increased susceptibility to RNS due to lower production of NADPH and mitochondrial disorders [46]. In agreement, a favohemoglobin denitrosylase (whose activity depends on NADPH) is important for detoxification of RNS [47,48].

Virulence factors

The definition of virulence factors is complex and very heterogeneous according to the source, but there is consensus that they can be defined as those elements of a pathogen that can cause damage in the host [49]. Following this definition, C. neoformans produces several degrading enzymes, such as proteases and lipases as virulence factors. These enzymes are also produced by other microbial pathogens, including fungi and bacteria [50–52]. In the case of C. neoformans, there is another degrading enzyme, urease, which also plays a role during infection. Urease catalyzes the degradation urea into CO₂ and ammonia, and is required for nitrogen utilization in multiple organisms. Cryptococcus neoformans produces very high amounts of urease, and it presence has been used as a diagnostic tool for cryptococcosis [53]. Urease is considered a virulence factor. Absence of this enzyme results in a fitness defect at slightly basic pH [54]. During infection, urease is required for brain invasion [55]. Urease promotes sequestration of cryptococcal cells at microcapillary vessels [56], and it has been hypothesized that ammonia promotes adhesion of C. neoformans either by increasing the expression of adhesins on the endothelia or by a direct toxic effect on the integrity tight junctions of the brain blood barrier (BBB) that would facilitate the brain invasion [56,57]. In agreement, proteins required for urease activity are also defective in brain invasion [58].

However, C. neoformans is well known because it expresses two clear virulence factors that interfere with the host immune systems, which are the capsule and melanin. Interestingly, these two components have a dual role, not only as virulence factors, but also as protective elements against some attacks of the immune response.

The polysaccharide capsule

The capsule is the structure that has been characterized in C. neoformans in most detail. It is mainly composed of two types of polysaccharides: glucuronoxylomanann (GXM) and glucuronoxylomannogalactan (GXMGal) [59–62]. There are other minor components, which are mannanproteins and chitin-like structures [63–65], but their function and role in capsule structure is still unknown.

Capsule structure is complex, and interestingly, it varies according to the environmental conditions. It is organized in branched fibers that attach to the cell wall and bind to each other through non covalent bonds [66,67].

Capsule synthesis is one of the topics of great interest in the biology of this pathogen (see reviews in Refs. [68–71]). Many of the proteins required for capsule synthesis are glycosidases that catalyze the interconversion of the different sugars that are bound to form the polysaccharide fibers (see reviews in Refs. [72–76]). However, there are still many aspects of capsule
synthesis that remain unknown. In the last years, there is increasing evidence that the polysaccharide components are synthesized in the ER and exported inside small vesicles [77]. The characterization of these vesicles has demonstrated that they contain, not only the capsular polysaccharide, but also other virulence factors that contribute through disease [78], and for example, they can influence the virulence of \textit{C. neoformans} in macrophages [79].

The capsule plays an important role in the virulence of \textit{C. neoformans}. However, it is not required for the regular life of the yeast since acapsular mutants can survive and replicate \textit{in vitro}, but it absence results in defects in virulence [80]. This is in part due to two main reasons. First, the capsule has some protective effects during infection. It has been shown that the capsular polysaccharide inhibits phagocytosis [81,82]. The most plausible explanation for this avoidance is that most epitopes that bind to the macrophage receptors (mainly cell wall components) are at the cell wall, and the presence of the capsule "hides" them from the phagocytic cell.

The capsule also confers protection against some stress factors, such as dehydration and free radicals [83–85]. The second function of the capsule during infection is its role as a true virulence factor. Many different studies have demonstrated that the capsular polysaccharides are secreted and have multiple effects on the host immune response (see reviews in Refs. [86,87]). Most data has been focused on GXM because it is the most abundant component of the capsule. This polysaccharide can inhibit neutrophil migration in different ways. GXM inhibits the exit of leukocytes from the blood vessels because it exhibits chemotaxing properties [88] and decreases the expression of chemokine receptors [89]. In addition, it inhibits the binding of leukocytes to endothelium by inducing L-selectin and E-cadherin shedding from neutrophils [89–91] and also by binding to CD18 [92].

Early studies demonstrated that infection with capsular polysaccharide also produces an immunological unresponsiveness, which is mainly manifested by an inhibition in the production of antibodies [93,94]. Later on, many different deleterious effects have been attributed to these polysaccharides. Among others, both GXM and GalGXM alter the cytokine production and affects maturation of dendritic cells and antigen presentation [95–99]. Another interesting mechanism involved in immune unresponsiveness is the effect of the capsular polysaccharides on the viability of some leukocytes. It has been shown that both GXM and GalGXM are potent inducers of apoptosis [100–103], which adds another role for the capsule as virulence factor.

The capsule can also interfere with elements of the host innate immune system. The polysaccharide inhibits the agglutination of cryptococcal cells by the surfactant protein SP-D. It is also noteworthy that SP-D\textsuperscript{−/−} mice are paradoxically more resistant to infection [104,105], which suggests that \textit{C. neoformans} has developed a mechanism to induce protective responses through some of the surfactant proteins.

\textbf{Melanin}

The other typical phenotypic feature of \textit{C. neoformans} is the accumulation of melanin, which is a dark pigment abundantly found in animals and fungi. In the case of \textit{C. neoformans}, melanin is only produced in the presence of exogenous compounds, mainly diphenolic compounds as L-DOPA [106,107]. Melanin synthesis depends on an enzyme, diphenol oxidase, encoded by two genes, \textit{LAC1} and \textit{LAC2}, being Lac1 the main protein involved in the production of this pigment [108].

Melanin plays a profound effect in the biology and virulence of \textit{C. neoformans}. Mutants unable to melanize present a significant reduction in their virulence [109,110]. It is well known that this pigment confers resistance to multiple stress factors, such as free radicals, ionizing radiation, and heat [111–113]. It can also bind and decrease the susceptibility to antifungal drugs [114,115]. Interestingly, a recent study has demonstrated that cryptococcal melanin enhances heat capture and contributes in this way to growth at low temperature [116].

In addition to its protective role, melanin has been involved in other processes required for virulence, and for this reason, is considered a true virulence factor too. Melanin seems to play a key role in the dissemination from the lung to the brain [117]. Melanization also changes the host cytokine production [118] and protects against macrophages [119]. The role of melanin as virulence factor has been established from the study of melanin particles (denominated melanin ghosts). Injection of mice with purified cryptococcal melanin [120] results in the formation of granulomas in the spleen, lung, and liver, indicating that this pigment can induce pro-inflammatory responses that alter the host immune response.

\textbf{Mechanisms for evasion of host immune response}

\textit{Cryptococcus neoformans} is a very particular yeast because it has developed some specific mechanisms that allow the evasion of some of the main attacks of the immune response, and in consequence, persist in tissues and organs for long periods.
Phagocytosis avoidance and intracellular pathogenesis

One of the areas characterized in most detail is the interaction of *C. neoformans* with phagocytic cells. After inhalation, spores and infectious particles have to face a layer of alveolar macrophages, which poses the first defense line in this organ. Although the capsule inhibits phagocytosis (see above), cryptococcal cells can in fact be phagocytosed in the presence of opsonins, mainly complement proteins and circulating antibodies. Even inside the macrophage, *C. neoformans* can survive and avoid killing [121,122]. This yeast is, in consequence, considered a facultative intracellular fungal pathogen because it can replicate inside the phagocytic cell and live in this niche for long time periods. At the moment, there are several responses that allow the intracellular survival. Cryptococcal cells inhibit the full acidification of the phagolysosome [123], which results is lower antimicrobial activity in this compartment. This inhibition is partially mediated by the activity of urease, which degrades urea into CO$_2$ and ammonia, which neutralizes the acidic pH of the phagolysosome [54]. In parallel, the phagolysosomes become leaky, which may also affect the functionality of the phagolysosome [122]. In agreement, it has been recently shown that interference of phagosome acidification of cryptococcal-infected macrophages correlates with higher replication of the fungal cells *in vivo* [124]. Finally, the capsule of *C. neoformans* has antioxidant properties [84], which also contributes to resistance to the free radicals produced in the phagolysosome. All these mechanisms are thought to be essential for the survival of cryptococcal cells in macrophages.

Another of the interesting research topics in the cryptococcal field is the outcome of yeast cells in the macrophages, and different processes might occur. For example, massive fungal replication might result in macrophage explosion. But yeast cells can be expelled from the macrophage through a process that does not affect the viability of the fungus nor the phagocytic cell. This process has been denominated as vomocytosis or non-lytic exocytosis [125,126]. Similarly, the cryptococcal cells can be transferred between macrophages [127,128]. Even depending in the activation state of the macrophage, the yeast cells can be efficiently destroyed and eliminated. And to make the situation more complex, several mechanisms might occur at the same time. For example, as observed in Supplemental Video 1, some yeast cells can replicate within a macrophage at the same time that other cryptococcal cells are attacked and eliminated in the same phagocytic cell.

In summary, the outcome of the interaction between *C. neoformans* and phagocytic cells is very complex, and many situations are possible. At the moment, we know some the factors that influence the fate of cryptococcal cells in macrophages, so more work is required to fully characterize this process, because it is believed that the result of this interaction is important to understand many aspects of the virulence of *C. neoformans*.

Capsular phenotypic variations

*Cryptococcus neoformans* has developed other mechanisms that contribute to the evasion of the host immune system, which are related to changes in some phenotypic features at the capsule and in the cell size of the yeast.

The capsule of *C. neoformans* is very dynamic, and it can undergo different rearrangements during infection (see reviews in Refs. [129,130]. In particular, there are three typical changes that have been described in this structure. First, the capsule can change in size. Early studies described that several factors can induce capsule growth in vitro, such as CO$_2$, iron limitation, mammalian serum, and nutrient limitation at basic pH [131–134]. More importantly, capsular enlargement is one of the first responses induced by *C. neoformans* in different types of hosts, including mammals, amoebas, and insects [135–137]. This process is important for the survival of the yeast during infection and to avoid the immune response. It has been shown that cells with enlarged capsule are more resistant to oxidative stress, antimicrobial peptides, and antifungals [84]. Furthermore, capsule growth also impairs phagocytosis mediated by complement [138]. In agreement, there is a correlation between the size of the capsule of the yeasts in the CSF and the intracranial pressure of patients with meningoencephalitis [139], suggesting that this process participates in the disease caused by this pathogen.

The capsule of the *C. neoformans* can also change its epitope structure both in vitro and during infection [140,141]. The importance of this process in the virulence has not been fully elucidated, but it has been correlated with the dissemination of the yeasts to the brain during infection [141]. Changes in the structure could also contribute to “hide” the fungal cells from elements of the immune system, such as circulating antibodies.

The third typical capsular change is related to the amount of polysaccharide and the density of this structure [142,143]. In parallel to capsule growth, the density of the polysaccharide capsule increases gradually during time *in vitro* [143]. This process correlates with the age of the cell. And in agreement, fungal cells isolated from *in vivo* have a more dense capsule due to a higher accumulation of polysaccharide fibers [142]. Increases
in capsule density are believed to contribute to immune evasion by hindering the penetration of molecules, such as antimicrobial peptides, and by inducing protection against other stress factors, such as free radicals.

**Cryptococcal morphogenesis and immune evasion**

A characteristic feature of fungi is the ability to grow in different morphological forms. The process that is more widely described is the formation of filaments, such as hyphae and pseudohyphae. In the case of fungi that can cause disease, the induction of morphological changes contributes to some important virulence mechanisms, such as adhesion and penetration into mucosae and tissues, and dissemination through the body [144–146]. Morphogenesis in *C. neoformans* is very peculiar and characteristic. This fungus can form pseudohyphae [147,148], but their exact role during infection remains to be fully understood. In contrast, there is a very unique and typical change induced in vivo, which involves a significant increase in the cell size. While cryptococcal cells in vitro have a size around 4–6 microns, in vivo the cell body can reach up to 40–50 microns [135,149,150]. This change is even more dramatic if the size of the capsule is considered, finding yeast cells of a total size that can reach up to 70–100 microns. These cells have been denominated as “titan cells” [151]. Due to their large size, the appearance of these cells results in a fungal population that cannot be easily eliminated by the immune system. Titan cells cannot be phagocytosed, are more resistant to stress factors and in consequence, contribute to fungal persistence during long time periods [149,150,152]. But there is also evidence that titan cells can also actively contribute to some important virulence mechanisms. For example, although due to their size these cells cannot invade nor penetrate biological barriers, titan cells can replicate and originate a progeny of daughter cells of regular size that can disseminate to other organs [153]. Titan cells can also impair the phagocytosis of yeast cells of regular size. Furthermore, the appearance of titan cells also seems to induce changes in the host immune response and cause a Th2 polarization [154,155], which are associated with non-protective responses during cryptococcosis.

Research on titan cells has been limited due to the difficulty to obtain them in vitro, and most knowledge about this transition has been obtained from the characterization of cells isolated from infected mice. However, recently, three different groups have identified conditions that mimic in vitro the formation of titan cells [156–158]. These conditions include incubation in nutrient limited media, serum, low cell density, and oxygen limitation. These articles have highlighted new conditions that induce titanization in *C. neoformans*. Cellular growth is enhanced when the cultures are inoculated with a low cell density, suggesting that the formation of titan cells is negatively regulated by quorum sensing molecules. In agreement, addition of Qsp1 (which is a short peptide that regulates QS in this pathogen [159,160]) inhibits titan cells formation [156,157], and deletion of the encoding gene results in an increase in cell size during hypoxia [156]. Another factor that induces titanization is addition of serum [157,158]. The central pathway involved in the development of titan cells depends on cAMP [150,157,158,161,162]. Several upstream and downstream elements on this pathway have been shown to regulate titanization in *C. neoformans*. Among them, the Ste3a pheromone receptor and the G-protein-coupled receptor Gpr5 are needed for titan cell formation, and both of them activate cAMP synthesis through the G-protein Gpa1 [149,161]. Furthermore, the Rim101 transcription factor, which is regulated by cAMP, is also required for titanization [161].

In summary, the discovery of in vitro conditions that produce the appearance of titan cells will greatly contribute in the future to understand the exact role of these cells during infection.

**Dissemination to the brain**

Despite survival in the lung is important in the virulence of *C. neoformans*, the dissemination from this organ through the organism and in particular to the brain. For still unknown reasons, this fungus has special tropism for the brain, where it causes meningoencephalitis. For this reason, the mechanisms that cause the dissemination and invasion of the BBB and later survival in the brain have been a priority in cryptococcal research [163,164]. And in fact, there is evidence that *C. neoformans* can invade the brain by different and concomitant mechanisms.

At the moment, three different ways of dissemination and BBB invasion have been proposed. Cryptococcal cells can bind to the luminal side of the BBB and be endocytosed by the endothelial cells [165,166]. Binding and invasion depends on host elements (such as CD44 and annexin A2 [167,168]) and cryptococcal factors, such as urease, phospholipase B, secretion of hyaluronic acid, and some metalloproteases [56,169,170,171].

The second mechanisms involves damage of the tight junctions of the BBB and cross of the cryptococcal cells between the endothelial cells (paracellular passage) [56,141,168].
Finally, the third mechanism is related to the ability of *C. neoformans* to survive in phagocytic cells. The intracellular pathogenesis of *C. neoformans* can provide not only a mechanism of immune evasion, but also a way to disseminate through the organism “hidden” inside infected phagocytic cells, a process denominated as the “Trojan horse” dissemination mechanism. In the last years, different groups have accumulated strong evidence that support the Trojan horse hypothesis. Depletion of macrophages results in reduced fungal burden in the brain, and injection of mice with macrophages infected *ex vivo* with *C. neoformans* increases the number of yeast cells in this organ [172]. Furthermore, the Trojan Horse migration process has been also demonstrated *in vitro* using BBB models in transwell plates [173,174]. This migration is enhanced by some immune mediators, such as MCP-1, IFN-γ, and TNF-γ. Interestingly, mutants that are defective in dissemination can cross in vitro generated BBB as wild type strains, supporting the role of the Trojan Horse mechanism in cryptococcal dissemination [174].

**Conclusions**

*Cryptococcus neoformans* was first described as pathogen at the end of the 19th century, although the characterization of its virulence mechanisms has been mainly studied in the last four decades. Despite the great effort invested by the scientific community in this pathogen, there are still great challenges to manage and decrease its impact. An example is the characterization of the capsule. This is the structure best characterized in this yeast, but we still do not know how it is synthesized in detail. A similar situation occurs with other virulence traits, such as intracellular pathogenesis. This is a characteristic cryptococcal feature, but its full contribution to virulence still remains unknown. A key process of cryptococcal disease is the dissemination of the yeasts to the brain. In this topic, there have been importance advances, but there is still a need to develop strategies to inhibit this step and brain invasion. The current antifungal therapy for cryptococcal disease is very limited, since echinocandins are not active against *C. neoformans*, and treatment is based on amphotericin B and fluconazole. Even though Amphotericin B is very active, its toxicity limits its use. There are some liposomal formulations that reduce the negative effects of this antifungal, but their high prices difficult their application in developing countries. For these reasons, future research is still needed to understand the virulence of this fungal pathogen.

**Disclosure statement**

No potential conflict of interest was reported by the author.

**Funding**

O.Z. is funded by grants SAF2014-54336-R and SAF2017-86192-R1 from the Spanish Ministry for Economics, Industry and Competitiveness. The author has no conflict of interest to declare; Ministerio de Economía, Industria y Competitividad, Gobierno de España [SAF2017-86192-R1]; Ministerio de Economía, Industria y Competitividad, Gobierno de España [SAF2014-54336R].

**References**

[1] Brown GD, Denning DW, Gow NA, et al. Hidden killers: human fungal infections. *Sci Transl Med*. 2012;4:165rv13.

[2] Drgona L, Khachatryan A, Stephens J, et al. Clinical and economic burden of invasive fungal diseases in Europe: focus on pre-emptive and empirical treatment of *Aspergillus* and *Candida* species. *Eur J Clin Microbiol Infect Dis*. 2014;33:7–21.

[3] Menzin J, Meyers JL, Friedman M, et al. The economic costs to United States hospitals of invasive fungal infections in transplant patients. *Am J Infect Control*. 2011;39:e15–20.

[4] Casadevall A, Perfect JR. *Cryptococcus neoformans*. Washington DC: ASM Press; 1998.

[5] Heitman J, Kozel TR, Kwon-Chung KJ, et al. *Cryptococcus*. From human pathogen to model yeast. Washington (DC): ASM Press; 2011.

[6] Park BJ, Wannemuehler KA, Marston BJ, et al. Estimation of the current global burden of cryptococcal meningitis among persons living with HIV/AIDS. *AIDS*. 2009;23:525–530.

[7] Rajasingham R, Smith RM, Park BJ, et al. Global burden of disease of HIV-associated cryptococcal meningitis: an updated analysis. *Lancet Infect Dis*. 2017;17:873–881.

[8] Martin TR, Frevert CW Innate immunity in the lungs. *Proc Am Thoracic Soc* 2005; 2:403–411.

[9] Casadevall A, Steenbergen JN, Nosanchuk JD. 'Ready made' virulence and 'dual use' virulence factors in pathogenic environmental fungi—the *Cryptococcus neoformans* paradigm. *Curr Opin Microbiol*. 2003;6:332–337.

[10] Littman ML, Borok R. Relation of the pigeon to cryptococcosis: natural carrier state, heat resistance and survival of *Cryptococcus neoformans*. *Mycopathol Mycol Appl*. 1968;35:329–345.

[11] Neilson JB, Ivey MH, Bulmer GS. *Cryptococcus neoformans*: pseudohyphal forms surviving culture with *Acanthamoeba polyphaga*. *Infect Immun*. 1978;20:262–266.

[12] Steenbergen JN, Shuman HA, Casadevall A. *Cryptococcus neoformans* interactions with amoebae suggest an explanation for its virulence and intracellular pathogenic strategy in macrophages. *Proc Natl Acad Sci U S A*. 2001;98:15245–15250.
[13] Apidianakis Y, Rahme LG, Heitman J, et al. Challenge of Drosophila melanogaster with Cryptococcus neoformans and role of the innate immune response. Eukaryot Cell. 2004;3:413–419.

[14] Mylonakis E, Moreno R, El Khoury JB, et al. Galleria mellonella as a model system to study Cryptococcus neoformans pathogenesis. Infect Immun. 2005;73:3842–3850.

[15] Warpeha KM, Park YD, Williamson PR. Susceptibility of intact germinating Arabidopsis thaliana to human fungal pathogens Cryptococcus neoformans and C. gattii. Appl Environ Microbiol. 2013;79:2979–2988.

[16] Venn-Watson S, Daniels R, Smith C. Thirty year retrospective evaluation of pneumonia in a bottlenose dolphin Tursiops truncatus population. Dis Aquat Organ. 2012;99:237–242.

[17] Malik R, Martin P, Wigneys DJ, et al. Nasopharyngeal cryptococcosis. Aust Vet J. 1997;75:483–488.

[18] Steenbergen JN, Casadevall A. The origin and maintenance of virulence for the human pathogenic fungus Cryptococcus neoformans. Microbes Infect. 2003;5:667–675.

[19] Casadevall A. Amoeba provide insight into the origin of virulence in pathogenic fungi. Adv Exp Med Biol. 2012;710:1–10.

[20] Velagapudi R, Hsueh YP, Geunes-Boyer S, et al. Spores as infectious propagules of Cryptococcus neoformans. Infect Immun. 2009;77:4345–4355.

[21] Giles SS, Dagenais TR, Botts MR, et al. Elucidating the pathogenesis of spores from the human fungal pathogen Cryptococcus neoformans. Infect Immun. 2009;77:3491–3500.

[22] Botts MR, Hull CM. Dueling in the lung: how Cryptococcus spores race the host for survival. Curr Opin Microbiol. 2010;13:437–442.

[23] Esher SK, Zaragoza O, Alspaugh JA. Cryptococcal pathogenic mechanisms: a dangerous trip from the environment to the brain. Mem Inst Oswaldo Cruz. 2018;113:e180057.

[24] Kronstad J, Saikia S, Nielson ED, et al. Adaptation of Cryptococcus neoformans to mammalian hosts: integrated regulation of metabolism and virulence. Eukaryot Cell. 2012;11:109–118.

[25] Koszubowski L, Lee SC, Heitman J. Signalling pathways in the pathogenesis of Cryptococcus. Cell Microbiol. 2009;11:370–380.

[26] Robert VA, Casadevall A. Vertebrate endothermy restricts most fungi as potential pathogens. J Infect Dis. 2009;200:1623–1626.

[27] Bergman O, Casadevall A. Mammalian endothermy optimally restricts fungi and metabolic costs. MBio. 2010;1:e00212–10.

[28] Petzold EW, Himmelreich U, Mylonakis E, et al. Characterization and regulation of the trehalose synthesis pathway and its importance in the pathogenicity of Cryptococcus neoformans. Infect Immun. 2006;74:5877–5887.

[29] Giles SS, Batinic-Haberle I, Perfect JR, et al. Cryptococcus neoformans mitochondrial superoxide dismutase: an essential link between antioxidant function and high-temperature growth. Eukaryot Cell. 2005;4:46–54.

[30] Kraus PR, Fox DS, Cox GM, et al. The Cryptococcus neoformans MAP kinase Mpk1 regulates cell integrity in response to antifungal drugs and loss of calcineurin function. Mol Microbiol. 2003;48:1377–1387.

[31] Alspaugh JA, Cavallo LM, Perfect JR, et al. RAS1 regulates filamentation, mating and growth at high temperature of Cryptococcus neoformans. Mol Microbiol. 2000;36:352–365.

[32] Gerwien F, Skrahina V, Kasper L, et al. Metals in fungal virulence. FEMS Microbiol Rev. 2018;42:1–21. doi:ARTN fxu050.

[33] Kronstad JW, Hu G, Jung WH. An encapsulation of iron homeostasis and virulence in Cryptococcus neoformans. Trends Microbiol. 2013;21:457–465.

[34] Bairwa G, Hee Jung W, Kronstad JW. Iron acquisition in fungal pathogens of humans. Metallomics. 2017;9:215–227.

[35] Saikia S, Oliveira D, Hu G, et al. Role of ferric reductases in iron acquisition and virulence in the fungal pathogen Cryptococcus neoformans. Infect Immun. 2014;82:839–850.

[36] Cadieux B, Lian T, Hu G, et al. The mannoprotein cig1 supports iron acquisition from heme and virulence in the pathogenic fungus Cryptococcus neoformans. J Infect Dis. 2013;207:1339–1347.

[37] Selvig K, Alspaugh JA. pH response pathways in fungi: adapting to host-derived and environmental signals. Mycobiology. 2011;39:249–256.

[38] Ost KS, O’Meara TR, Huda N, et al. The Cryptococcus neoformans alkaline response pathway: identification of a novel rim pathway activator. PLoS Genet. 2015;11:e1005159.

[39] O’Meara TR, Holmer SM, Selvig K, et al. Cryptococcus neoformans rim101 is associated with cell wall remodeling and evasion of the host immune responses. MBio. 2013;4:e00522–12.

[40] O’Meara TR, Norton D, Price MS, et al. Interaction of Cryptococcus neoformans rim101 and protein kinase A regulates capsule. PLoS Pathog. 2010;6:e1000776.

[41] Ost KS, Esher SK, Leopold Wager CM, et al. Rim pathway-mediated alterations in the fungal cell wall influence immune recognition and inflammation. MBio. 2017;8:e02290–16.

[42] Missall TA, Cherry-Harris JF, Lodge JK. Two glutathione peroxidases in the fungal pathogen Cryptococcus neoformans are expressed in the presence of specific substrates. Microbiology. 2005;151:2573–2581.

[43] Missall TA, Lodge JK. Function of the thioredoxin proteins in Cryptococcus neoformans during stress or virulence and regulation by putative transcriptional modulators. Mol Microbiol. 2005;57:847–858.

[44] Missall TA, Moran JM, Corbett JA, et al. Distinct stress responses of two functional laccases in Cryptococcus neoformans are revealed in the absence of the thiol-specific antioxidant Tsa1. Eukaryot Cell. 2005;4:202–208.

[45] Chaturvedi V, Wong B, Newman SL. Oxidative killing of Cryptococcus neoformans by human neutrophils, evidence that fungal mannitol protects by scavenging reactive oxygen intermediates. J Immunol. 1996;156:3836–3840.
Brown SM, Upadhya R, Shoemaker JD, et al. Isocitrate dehydrogenase is important for nitrosative stress resistance in Cryptococcus neoformans, but oxidative stress resistance is not dependent on glucose-6-phosphate dehydrogenase. Eukaryot Cell. 2010;9:971–980.

Chow ED, Liu OW, O’Brien S, et al. Exploration of whole-genome responses of the human AIDS-associated yeast pathogen Cryptococcus neoformans var grubii: nitric oxide stress and body temperature. Curr Genet. 2007;52:137–148.

de Jesus-Berrios M, Liu L, Nussbaum JC, et al. Enzymes that counteract nitrosative stress promote fungal virulence. Curr Biol. 2003;13:1963–1968.

Pirofski LA, Casadevall A. What is infectiveness and how is it involved in infection and immunity? BMC Immunol. 2015;16:13.

Schaller M, Borelli C, Korting HC, et al. Hydrolytic enzymes as virulence factors of Candida albicans. Mycoses. 2005;48:365–377.

Singh G, Singh G, Jadeja D, et al. Lipid hydrolizing enzymes in virulence: Mycobacterium tuberculosis as a model system. Crit Rev Microbiol. 2010;36:259–269.

Toth R, Toth A, Vagvolgyi C, et al. Capsideparisiosis secreted lipase as an important virulence factor. Curr Protein Pept Sci. 2017;18:1043–1049.

Zimmer BL, Roberts GD. Rapid selective urease test for presumptive identification of Cryptococcus neoformans. J Clin Microbiol. 1979;10:380–381.

Fu MS, Coelho C, De Leon-Rodriguez CM, et al. Cryptococcus neoformans urease affects the outcome of intracellular pathogenesis by modulating phagolysosomal pH. PLoS Pathog. 2018;14:e1007144.

Cox GM, Mukherjee J, Cole GT, et al. Urease as a virulence factor in experimental cryptococcosis. Infect Immun. 2000;68:443–448.

Olszewski MA, Noverr MC, Chen GH, et al. Urease expression by Cryptococcus neoformans promotes microvascular sequestration, thereby enhancing central nervous system invasion. Am J Pathol. 2004;164:1761–1771.

Taylor-Robinson SD, Jackson N, Buckley C. Helicobacter pylori, ammonia and the brain. Gut. 1997;40:805–806.

Singh A, Panting RJ, Varma A, et al. Factors required for activation of urease as a virulence determinant in Cryptococcus neoformans. MBio. 2013;4:e00220–13.

Cherniak R, Jones RG, Reiss E. Structure determination of Cryptococcus neoformans serotype A-variant glucuronoxylomanan by 13C-n.m.r. spectroscopy. Carbohydr Res. 1988;172:113–138.

Murphy JW, Mosley RL, Cherniak R, et al. Serological, electrophoretic, and biological properties of Cryptococcus neoformans antigens. Infect Immun. 1988;56:424–431.

Cherniak R, Reiss E, Turner S, A galactoxylomannan antigen of Cryptococcus neoformans serotype A. Carbohydr Res. 1982;103:239–250.

Heiss C, Kluitts JS, Wang Z, et al. The structure of Cryptococcus neoformans galactoxylomannan contains beta-D-glucuronic acid. Carbohydr Res. 2009;344:915–920.

Murphy JW. Influence of cryptococcal antigens on cell-mediated immunity. Rev Infect Dis. 1988;10 (Suppl 2):S532–5.

Vartivarian SE, Reyes GH, Jacobson ES, et al. Localization of mannoprotein in Cryptococcus neoformans. J Bacteriol. 1989;171:6850–6852.

Rodrigues ML, Alvarez M, Fonseca FL, et al. Binding of the wheat germ lectin to Cryptococcus neoformans suggests an association of chitinlike structures with yeast budding and capsular glucuronoxylomanan. Eukaryot Cell. 2008;7:602–609.

Pierini LM, Doering TL. Spatial and temporal sequence of capsule construction in Cryptococcus neoformans. Mol Microbiol. 2001;41:105–115.

Frases S, Pontes B, Nimrichter L, et al. Capsule of Cryptococcus neoformans grows by enlargement of polysaccharide molecules. Proc Natl Acad Sci U S A. 2009;106:1228–1233.

Zaragoza O, Rodrigues ML, De Jesus M, et al. The capsule of the fungal pathogen Cryptococcus neoformans. Adv Appl Microbiol. 2009;68:133–216.

O’Meara TR, Alspaugh JA. The Cryptococcus neoformans capsule: a sword and a shield. Clin Microbiol Rev. 2012;25:387–403.

Doering TL. How does Cryptococcus get its coat? Trends Microbiol. 2000;8:547–553.

Doering TL. How sweet it is! Cell wall biogenesis and polysaccharide capsule formation in Cryptococcus neoformans. Annu Rev Microbiol. 2009;63:223–247.

Wang ZA, Li LX, Doering TL. Unraveling synthesis of the cryptococcal cell wall and capsule. Glycobiology. 2018;28:719–730.

Ding H, Mayer FL, Sanchez-Leon E, et al. Networks of fibers and factors: regulation of capsule formation in Cryptococcus neoformans. PLoS Pathog. 2016;5.

Agustinho DP, Miller LC, Li LX, et al. Peeling the onion: the outer layers of Cryptococcus neoformans. Mem Inst Oswaldo Cruz. 2018;113:e180040.

Casadevall A, Coelho C, Cordero RJB, et al. The capsule of Cryptococcus neoformans. Virulence. 2018;10–1.

Rodrigues ML, Casadevall A, Zaragoza O. The architecture and antigenic composition of the polysaccharide capsule. In: Heitman J, Kozel TR, Kwon-Chung KJ, et al., editors. Cryptococcus from human pathogen to model yeast. Washington (D.C): ASM Press; 2011. p. 43–54.

Rodrigues ML, Nimrichter L, Oliveira DL, et al. Vesicular polysaccharide export in Cryptococcus neoformans is an eukaryotic solution to the problem of fungal trans-cell wall transport. Eukaryot Cell. 2007;6:48–59.

Nimrichter L, de Souza MM, Del Poeta M, et al. Extracellular vesicle-associated transitory cell wall components and their impact on the interaction of fungi with host cells. Front Microbiol. 2016;7:1034.

Oliveira DL, Freire-de-Lima CG, Nosanchuk JD, et al. Extracellular vesicles from Cryptococcus neoformans modulate macrophage functions. Infect Immun. 2010;78:1601–1609.

Chang YC, Kwon-Chung KJ. Complementation of a capsule-deficient mutation of Cryptococcus
restores its virulence. Mol Cell Biol. 1994;14:4912–4919.

Kozel TR, Gotschlich EC. The capsule of Cryptococcus neoformans passively inhibits phagocytosis of the yeast by macrophages. J Immunol. 1982;129:1675–1680.

Kozel TR, Mastroianni RP. Inhibition of phagocytosis by cryptococcal polysaccharide: dissociation of the attachment and ingestion phases of phagocytosis. Infect Immun. 1976;14:62–67.

Aksenov SI, Babyeva IP, Golubev VI. On the mechanism of adaptation of micro-organisms to conditions of extreme low humidity. Life Sci Space Res. 1973;11:55–61.

Zaragoza O, Chrisman CJ, Castelli MV, et al. Capsule enlargement in Cryptococcus neoformans confers resistance to oxidative stress suggesting a mechanism for intracellular survival. Cell Microbiol. 2008;10:2043–2057.

Cordero RJ, Frases S, Guimaraes AJ, et al. Evidence for branching in cryptococcal capsular polysaccharides and consequences on its biological activity. Mol Microbiol. 2011;79:1101–1117.

Vecchiarelli A, Pericolini E, Gabrielli E, et al. Elucidating the immunological function of the Cryptococcus neoformans capsule. Future Microbiol. 2013;8:1107–1116.

Vecchiarelli A. Immunoregulation by capsular components of Cryptococcus neoformans. Med Mycol. 2000;38:407–417.

Dong ZM, Murphy JW. Mobility of human neutrophils in response to Cryptococcus neoformans cells, culture filtrate antigen, and individual components of the antigen. Infect Immun. 1993;61:5067–5077.

Dong ZM, Murphy JW. Cryptococcal polysaccharides induce L-selectin shedding and tumor necrosis factor receptor loss from the surface of human neutrophils. J Clin Invest. 1996;97:689–698.

Ellerbroek PM, Hoepelman AI, Wolbers F, et al. Cryptococcal glucuronoxylomannan inhibits adhesion of neutrophils to stimulated endothelium in vitro by affecting both neutrophils and endothelial cells. Infect Immun. 2002;70:4762–4771.

Ellerbroek PM, Ulfman LH, Hoepelman AI, et al. Cryptococcal glucuronoxylomannan interferes with neutrophil rolling on the endothelium. Cell Microbiol. 2004;6:581–592.

Dong ZM, Murphy JW. Cryptococcal polysaccharides bind to CD18 on human neutrophils. Infect Immun. 1997;65:557–563.

Blackstock R, Hall NK. Non-specific immunosuppression by Cryptococcus neoformans infection. Mycopathologia. 1984;86:35–43.

Murphy JW, Cozad GC. Immunological unresponsiveness induced by cryptococcal capsular polysaccharide assayed by the hemolytic plaque technique. Infect Immun. 1972;5:896–901.

Vecchiarelli A, Pericolini E, Gabrielli E, et al. Cryptococcus neoformans galactoxylomannan is a potent negative immunomodulator, inspiring new approaches in anti-inflammatory immunotherapy. Immunotherapy. 2011;3:997–1005.

Blackstock R. Cryptococcal capsular polysaccharide utilizes an antigen-presenting cell to induce a T-suppressor cell to secrete TsF. J Med Vet Mycol. 1996;34:19–30.

Blackstock R, Casadevall A. Presentation of cryptococcal capsular polysaccharide (GXM) on activated antigen-presenting cells inhibits the T-suppressor response and enhances delayed-type hypersensitivity and survival. Immunology. 1997;92:334–339.

Vecchiarelli A. The cellular responses induced by the capsular polysaccharide of Cryptococcus neoformans differ depending on the presence or absence of specific protective antibodies. Curr Mol Med. 2005;5:413–420.

Chiarello LS, Baroni TL, Aoki MP, et al. Immunosuppression, interleukin-10 synthesis and apoptosis are induced in rats inoculated with Cryptococcus neoformans glucuronoxylomannan. Immunology. 2004;113:392–400.

De Jesus M, Nicola AM, Frases S, et al. Galactoxylomannan-mediated immunological paralysis results from specific B cell deletion in the context of widespread immune system damage. J Immunol. 2009;183:3885–3894.

Chiarello LS, Baroni TL, Garro AP, et al. Cryptococcus neoformans glucuronoxylomannan induces macrophage apoptosis mediated by nitric oxide in a caspase-independent pathway. Int Immunol. 2008;20:1527–1541.

Monari C, Pericolini E, Bistoni G, et al. Cryptococcus neoformans capsular glucuronoxylomannan induces expression of fas ligand in macrophages. J Immunol. 2005;174:3461–3468.

Villena SN, Pinheiro RO, Pinheiro CS, et al. Capsular polysaccharides galactoxylomannan and glucuronoxylomannan from Cryptococcus neoformans induce macrophage apoptosis mediated by Fas ligand. Cell Microbiol. 2008;10:1274–1285.

Geunes-Boyé S, Beers MF, Perfect JR, et al. Surfactant protein D facilitates Cryptococcus neoformans infection. Infect Immun. 2012;80:2444–2453.

Holmer SM, Evans KS, Asfaw YG, et al. Impact of surfactant protein D, interleukin-5, and eosinophilia on Cryptococcosis. Infect Immun. 2014;82:683–693.

Chaske S, Tyndall RL. Pigment production by Cryptococcus neoformans and other Cryptococcus species from aminophenols and diaminobenzenes. J Clin Microbiol. 1978;7:146–152.

Nurudeen TA, Ahearn DG. Regulation of melanin production by Cryptococcus neoformans. J Clin Microbiol. 1979;10:724–729.

Pukkila-Worley R, Geralrd QD, Kraus PR, et al. Transcriptional network of multiple capsule and melanin genes governed by the Cryptococcus neoformans cyclic AMP cascade. Eukaryot Cell. 2005;4:190–201.

Kwon-Chung KJ, Polacheck I, Popkin TJ. Melanin-lacking mutants of Cryptococcus neoformans and their virulence for mice. J Bacteriol. 1982;150:1414–1421.

Salas SD, Bennett JE, Kwon-Chung KJ, et al. Effect of the laccase gene CNLAC1 on virulence of Cryptococcus neoformans. J Exp Med. 1996;184:377–386.
[111] Rosas AL, Casadevall A. Melanization affects susceptibility of Cryptococcus neoformans to heat and cold. FEMS Microbiol Lett. 1997;153:265–272.

[112] Wang Y, Casadevall A. Decreased susceptibility of melanized Cryptococcus neoformans to UV light. Appl Environ Microbiol. 1994;60:3864–3866.

[113] Nosanchuk JD, Casadevall A. Impact of melanin on microbial virulence and clinical resistance to antimicrobial compounds. Antimicrob Agents Chemother. 2006;50:3519–3528.

[114] Wang Y, Casadevall A. Growth of Cryptococcus neoformans in presence of L-Dopa decreases its susceptibility to amphotericin B. Antimicrob Agents Chemother. 1994;38:2648–2650.

[115] van Duin D, Casadevall A, Nosanchuk JD. Melanization of Cryptococcus neoformans and Histoplasma capsulatum reduces their susceptibilities to amphotericin B and caspofungin. Antimicrob Agents Chemother. 2002;46:3394–3400.

[116] Cordero RJB, Robert V, Cardinàlì G, et al. Impact of yeast pigmentation on heat capture and latitudinal distribution. Curr Biol. 2018;28:2657–64 e3.

[117] Noverr MC, Williamson PR, Fajardo RS, et al. CNLAC1 is required for extrapulmonary dissemination of Cryptococcus neoformans but not pulmonary persistence. Infect Immun. 2004;72:1693–1699.

[118] Mednick AJ, Nosanchuk JD, Casadevall A. Melanization of Cryptococcus neoformans affects lung inflammatory responses during cryptococcal infection. Infect Immun. 2005;73:2012–2019.

[119] Liu L, Tewari RP, Williamson PR. Laccase protects Cryptococcus neoformans from antifungal activity of alveolar macrophages. Infect Immun. 1999;67:6034–6039.

[120] Rosas AL, MacGill RS, Nosanchuk JD, et al. Activation of the alternative complement pathway by fungal melanins. Clin Diagn Lab Immunol. 2002;9:144–148.

[121] Diamond RD, Bennett JE. Growth of Cryptococcus neoformans within human macrophages in vitro. Infect Immun. 1973;7:231–236.

[122] Tucker SC, Casadevall A. Replication of Cryptococcus neoformans in macrophages is accompanied by phagosomal permeabilization and accumulation of vesicles containing polysaccharide in the cytoplasm. Proc Natl Acad Sci U S A. 2002;99:3165–3170.

[123] Smith LM, Dixon EF, May RC. The fungal pathogen Cryptococcus neoformans manipulates macrophage phagosome maturation. Cell Microbiol. 2015;17:702–713.

[124] De Leon-Rodriguez CM, Rossi DCP, Fu MS, et al. The outcome of the Cryptococcus neoformans-macrophage interaction depends on phagolysosomal membrane integrity. J Immunol. 2018;201:583–603.

[125] Ma H, Croudace JE, Lammas DA, et al. Expulsion of live pathogenic yeast by macrophages. Curr Biol. 2006;16:2156–2160.

[126] Alvarez M, Casadevall A. Phagosome extrusion and host-cell survival after Cryptococcus neoformans phagocytosis by macrophages. Curr Biol. 2006;16:2161–2165.

[127] Alvarez M, Casadevall A. Cell-to-cell spread and massive vacuole formation after Cryptococcus neoformans infection of murine macrophages. BMC Immunol. 2007;8:16.

[128] Ma H, Croudace JE, Lammas DA, et al. Direct cell-to-cell spread of a pathogenic yeast. BMC Immunol. 2007;8:15.

[129] McFadden D, Zaragoza O, Casadevall A. The capsular dynamics of Cryptococcus neoformans. Trends Microbiol. 2006;14:497–505.

[130] Zaragoza O. Multiple disguises for the same party: the concepts of morphogenesis and phenotypic variations in Cryptococcus neoformans. Front Microbiol. 2011;2:181.

[131] Vartivarian SE, Anaissie EJ, Cowart RE, et al. Regulation of cryptococcal capsular polysaccharide by iron. J Infect Dis. 1993;167:186–190.

[132] Granger DL, Perfect JR, Durack DT. Virulence of Cryptococcus neoformans. regulation of capsule synthesis by carbon dioxide. J Clin Invest. 1985;76:508–516.

[133] Zaragoza O, Fries BC, Casadevall A. Induction of capsule growth in Cryptococcus neoformans by mammalian serum and CO(2). Infect Immun. 2003;71:6155–6164.

[134] Zaragoza O, Casadevall A. Experimental modulation of capsule size in Cryptococcus neoformans. Biol Proced Online. 2004;6:10–15.

[135] Feldmesser M, Kress Y, Casadevall A. Dynamic changes in the morphology of Cryptococcus neoformans during murine pulmonary infection. Microbiology. 2001;147:2355–2365.

[136] Chrisman CJ, Albuquerque P, Guimaraes AJ, et al. Phospholipids trigger Cryptococcus neoformans capsular enlargement during interactions with amoebae and macrophages. PLoS Pathog. 2011;7:e1002047.

[137] García-Rodas R, Casadevall A, Rodriguez-Tudela JL, et al. Cryptococcus neoformans capsular enlargement and cellular gigantism during Galleria mellonella infection. PLoS One. 2011;6:e24485.

[138] Zaragoza O, Taborda CP, Casadevall A. The efficacy of complement-mediated phagocytosis of Cryptococcus neoformans is dependent on the location of C3 in the polysaccharide capsule and involves both direct and indirect C3-mediated interactions. Euro J Immunol. 2003;33:1957–1967.

[139] Robertson EJ, Najjuka G, Rolfes MA, et al. Cryptococcus neoformans ex vivo capsule size is associated with intracranial pressure and host immune response in HIV-associated cryptococcal meningitis. J Infect Dis. 2014;209:74–82.

[140] McFadden DC, Fries BC, Wang F, et al. Capsule structural heterogeneity and antigenic variation in Cryptococcus neoformans. Eukaryot Cell. 2007;6:1464–1473.

[141] Charlier C, Chretien F, Baudrimont M, et al. Capsule structure changes associated with Cryptococcus neoformans crossing of the blood-brain barrier. Am J Pathol. 2005;166:421–432.

[142] Gates MA, Thorkildson P, Kozel TR. Molecular architecture of the Cryptococcus neoformans capsule. Mol Microbiol. 2004;52:13–24.

[143] Maxson ME, Dadachova E, Casadevall A, et al. Radial mass density, charge, and epitope distribution in the Cryptococcus neoformans capsule. Eukaryot Cell. 2007;6:95–109.

[144] Romani L, Bistoni F, Puccetti P. Adaptation of Candida albicans to the host environment: the role
of morphogenesis in virulence and survival in mammalian hosts. Curr Opin Microbiol. 2003;6:338–343.

[145] San-Blas G, Travassos LR, Fries BC, et al. Fungal morphogenesis and virulence. Med Mycol. 2000;38 (Suppl 1):79–86.

[146] Trevijano-Contador N, Rueda C, Zaragoza O. Fungal morphogenetic changes inside the mammalian host. Semin Cell Dev Biol. 2016;57:100–109.

[147] Lee SC, Phadke S, Sun S, et al. Pseudohyphal growth of Cryptococcus neoformans is a reversible dimorphic transition in response to ammonium that requires Amt1 and Amt2 ammonium permeases. Eukaryot Cell. 2012;11:1391–1398.

[148] Lin J, Idnurm A, Lin X. Morphology and its underlying genetic regulation impact the interaction between Cryptococcus neoformans and its hosts. Med Mycol. 2015;53:493–504.

[149] Okagaki LH, Strain AK, Nielsen JN, et al. Cryptococcal cell morphology affects host cell interactions and pathogenicity. PLoS Pathog. 2010;6:e1000953.

[150] Zaragoza O, Garcia-Rodas R, Nosanchuk JD, et al. Fungal cell gigantism during mammalian infection. PLoS Pathog. 2010;6:e1000945.

[151] Zaragoza O, Nielsen K. Titan cells in Cryptococcus neoformans: cells with a giant impact. Curr Opin Microbiol. 2013;16:409–413.

[152] Okagaki LH, Nielsen K. Titan cells confer protection from phagocytosis in Cryptococcus neoformans infections. Eukaryot Cell. 2012;11:820–826.

[153] Gerstein AC, Fu MS, Mukaremera L, et al. Polyploid titan cells produce haploid and aneuploid progeny to promote stress adaptation. MBio. 2015;6:e01340–15.

[154] Crabtree JN, Okagaki LH, Wiesner DL, et al. Titan cell production enhances the virulence of Cryptococcus neoformans. Infect Immun. 2012;80:3776–3785.

[155] Garcia-Barbazan I, Trevijano-Contador N, Rueda C, et al. The formation of titan cells in Cryptococcus neoformans depends on the mouse strain and correlates with induction of Th2-type responses. Cell Microbiol. 2016;18:111–124.

[156] Hommel B, Mukaremera L, Cordero RJB, et al. Titan cells formation in Cryptococcus neoformans is finely tuned by environmental conditions and modulated by positive and negative genetic regulators. PLoS Pathog. 2018;14:e1006982.

[157] Trevijano-Contador N, de Oliveira HC, Garcia-Rodas R, et al. Cryptococcus neoformans can form titan-like cells in vitro in response to multiple signals. PLoS Pathog. 2018;14:e1007007.

[158] Dambuza IM, Drake T, Chapuis A, et al. The Cryptococcus neoformans titan cell is an inducible and regulated morphotype underlying pathogenesis. PLoS Pathog. 2018;14:e1006978.

[159] Homer CM, Summers DK, Goranov AI, et al. Intracellular action of a secreted peptide required for fungal virulence. Cell Host Microbe. 2016;19:849–864.

[160] Lee H, Chang YC, Nardone G, et al. TUP1 disruption in Cryptococcus neoformans uncovers a peptide-mediated density-dependent growth phenomenon that mimics quorum sensing. Mol Microbiol. 2007;64:591–601.

[161] Okagaki LH, Wang Y, Ballou ER, et al. Cryptococcal titan cell formation is regulated by G-protein signaling in response to multiple stimuli. Eukaryot Cell. 2011. Epub ahead of print

[162] Choi J, Vogl AW, Kronstad JW. Regulated expression of cyclic AMP-dependent protein kinase A reveals an influence on cell size and the secretion of virulence factors in Cryptococcus neoformans. Mol Microbiol. 2012;85:700–715.

[163] Tseng HK, Huang TY, Wu AY, et al. How Cryptococcus interacts with the blood-brain barrier. Future Microbiol. 2015;10:1669–1682.

[164] Colombo AC, Rodrigues ML. Fungal colonization of the brain: anatopathological aspects of neurological cryptococcosis. An Acad Bras Cienc. 2015;87:1293–1309.

[165] Chen SH, Stins MF, Huang SH, et al. Cryptococcus neoformans induces alterations in the cytoskeleton of human brain microvascular endothelial cells. J Med Microbiol. 2003;52:961–970.

[166] Shi M, Cologusso P, Mody CH. Real-time in vivo imaging of fungal migration to the central nervous system. Cell Microbiol. 2012;14:1819–1827.

[167] Jong A, Wu CH, Shackelford GM, et al. Involvement of human CD44 during Cryptococcus neoformans infection of brain microvascular endothelial cells. Cell Microbiol. 2008;10:1313–1326.

[168] Vu K, Eigenheer RA, Phinney BS, et al. Cryptococcus neoformans promotes its transmigration into the central nervous system by inducing molecular and cellular changes in brain endothelial cells. Infect Immun. 2013;81:3139–3147.

[169] Liu TB, Kim JC, Wang Y, et al. Brain inositol is a novel stimulator for promoting Cryptococcus penetratin of the blood-brain barrier. PLoS Pathog. 2013;9:e1003247.

[170] Santangelo R, Zoellner H, Sorrell T, et al. Role of extracellular phospholipases and mononuclear phagocytes in dissemination of cryptococcosis in a murine model. Infect Immun. 2004;72:2229–2239.

[171] Na Pombejra S, Salemi M, Phinney BS, et al. The metalloprotease, Mpr1, engages annexinA2 to promote the transcytosis of fungal cells across the blood-brain barrier. Front Cell Infect Microbiol. 2017;7:296.

[172] Charlier C, Nielsen K, Daou S, et al. Evidence for TUP1 transcription in brain: anatomopathological aspects of neurological cryptococcosis. An Acad Bras Cienc. 2015;87:1293–1309.

[173] Chen SH, Stins MF, Huang SH, et al. Cryptococcus neoformans induces alterations in the cytoskeleton of human brain microvascular endothelial cells. J Med Microbiol. 2003;52:961–970.

[174] Shi M, Cologusso P, Mody CH. Real-time in vivo imaging of fungal migration to the central nervous system. Cell Microbiol. 2012;14:1819–1827.

[175] Jong A, Wu CH, Shackelford GM, et al. Involvement of human CD44 during Cryptococcus neoformans infection of brain microvascular endothelial cells. Cell Microbiol. 2008;10:1313–1326.

[176] Vu K, Eigenheer RA, Phinney BS, et al. Cryptococcus neoformans promotes its transmigration into the central nervous system by inducing molecular and cellular changes in brain endothelial cells. Infect Immun. 2013;81:3139–3147.

[177] Liu TB, Kim JC, Wang Y, et al. Brain inositol is a novel stimulator for promoting Cryptococcus penetratin of the blood-brain barrier. PLoS Pathog. 2013;9:e1003247.

[178] Santangelo R, Zoellner H, Sorrell T, et al. Role of extracellular phospholipases and mononuclear phagocytes in dissemination of cryptococcosis in a murine model. Infect Immun. 2004;72:2229–2239.

[179] Na Pombejra S, Salemi M, Phinney BS, et al. The metalloprotease, Mpr1, engages annexinA2 to promote the transcytosis of fungal cells across the blood-brain barrier. Front Cell Infect Microbiol. 2017;7:296.

[180] Charlier C, Nielsen K, Daou S, et al. Evidence for TUP1 transcription in brain: anatomopathological aspects of neurological cryptococcosis. An Acad Bras Cienc. 2015;87:1293–1309.