New Insights on the Pathogenesis of Invasive *Cryptococcus neoformans* Infection

Helene C. Eisenman, PhD, Arturo Casadevall, MD, PhD, and Erin E. McClelland, PhD

Corresponding author
Arturo Casadevall, MD, PhD
Albert Einstein College of Medicine, 411 Forchheimer,
1300 Morris Park Avenue, Bronx, NY 10461, USA.
E-mail: casadeva@aecom.yu.edu

Disseminated cryptococcosis begins with infection of the lungs via inhalation. This is followed by escape from the lungs and entry into the bloodstream allowing dissemination to the brain and central nervous system. We discuss the steps involved in dissemination and the host and microbial factors that influence each step. For the host, containment in the lung is accomplished with a combination of cell-mediated and antibody responses. Dissemination occurs when these systems fail and/or when phagocytic cells that fail to kill the yeast instead act as a niche for replication. One of the main microbial factors affecting dissemination is the polysaccharide capsule, a major virulence factor that promotes dissemination at every step. Secreted enzymes are important, including laccase and phospholipase B, which promote escape from the lungs, and urease, which contributes to crossing the blood–brain barrier. Lastly, a number of regulatory factors contribute, especially to growth of *Cryptococcus neoformans* in the brain.

Introduction

For a host who has inhaled the opportunistic yeast *Cryptococcus neoformans* (*Cn*), the defining event is whether infection is cleared, remains confined to the lung, or undergoes extrapulmonary dissemination. The mechanism of dissemination is thought to involve the following steps: 1) growth in the lung and crossing the bronchoalveolar epithelium to enter the lymphatic system for subsequent entry into the circulation or direct invasion of the bloodstream, 2) escape from intravascu-lar mechanisms that kill yeast during transport through the blood, 3) infection of the central nervous system (CNS) by crossing the blood–brain barrier (BBB), and 4) growth in the brain [1,2•,3•]. We discuss how the host and microbial factors are involved at each step in dissemination (Table 1).

Growth in the Lung and Exit into the Bloodstream or Lymphatic System

Host factors

Containment of the microbe in the lungs is crucial in host defense against cryptococcosis. Much of the research on *Cn* pathogenesis in the lung relies on using the murine infection model. Mice are usually infected with *Cn* via the intratracheal route to mimic the natural course of infection and generally succumb to cryptococcosis within weeks of infection. In contrast, lung containment ultimately leads to reduced dissemination to the brain and other organs. Containment of *Cn* in the lung requires an orchestrated immune response that relies on multiple components, including cell-mediated, antibody-based, and innate immune mechanisms.

Cell-mediated immunity, including both CD8+ and CD4+ T cells, especially of the Th1 class, is required to eliminate *Cn* from the lungs. The production of Th1 cytokines and chemokines, especially for the recruitment and activation of macrophages, is essential. Macrophages play a key role in the immune defense against *Cn*. Containment of the fungal cells in granulomas in the lung is generally associated with a positive outcome for the host (Fig. 1). In contrast, a Th2 response is associated with dissemination to the CNS and elsewhere with a negative outcome for the host. Thus, there are many possible targets of immunotherapy in treating cryptococcosis.

A number of Th1 cytokines are implicated in promoting a strong host defense in the lungs. For example, tumor necrosis factor (TNF)-α is produced in the lungs of infected mice within a few days of infection. Inactivation with a single dose of anti–TNF-α antibody on the day of infec-

Table 1. Microbial and host factors involved in dissemination

Stage of dissemination	Microbial factors enhancing dissemination	Host factors preventing dissemination
Growth in lung/exit from lung into bloodstream or lymphatic system	Capsule	Th1 CD8$^+$ T cells
	Laccase	Th1 CD4$^+$ T cells
	Phospholipase B	TNF-α
	Inositol phosphosphingolipid-phospholipase	IFN-γ
		IL-18
		Granulomas
		Antibodies
		Genetics of host
Survival in the bloodstream or lymphatic system	Capsule	Macrophages
Crossing the blood–brain barrier	Transcytosis	Macrophages
	Capsule	
	Urease	
Growth in brain	SRE1	Th1 cytokines
	CTR4	Th1 chemokines
		Genetics of host

IFN—interferon; IL—interleukin; SRE—sterol regulatory element-binding protein; Th—T-helper; TNF—tumor necrosis factor.
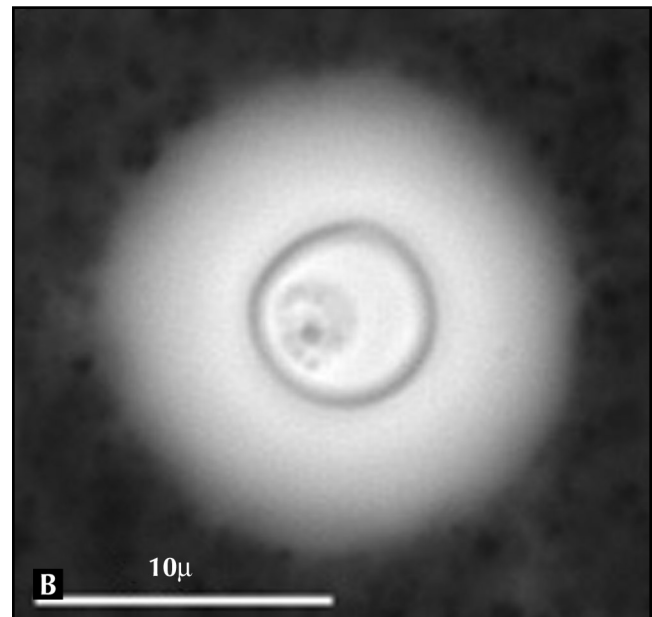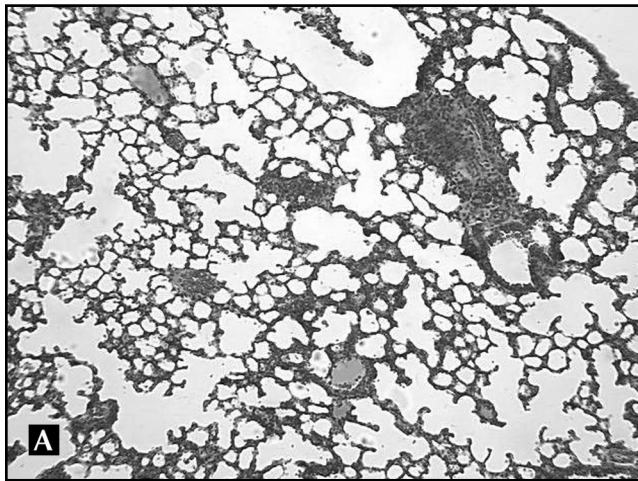

Figure 1. Key factors affecting dissemination of *Cn*. **A,** Granuloma in the lung. Containment of the microbe in the lung is crucial to preventing disseminated infection. **B,** India ink stain of *Cn* showing the capsule surrounding the cell wall. The capsule has many immunomodulatory effects that protect the microbe from the host immune system.

tion is sufficient to increase pulmonary fungal burden and dissemination of *Cn* to the brain and spleen [4]. Interferon (IFN)-γ is another important Th1 cytokine. Inactivating IFN-γ with antibodies worsens infection, as assessed by decreased survival and increased fungal burden in the lung and brain. In contrast, administering recombinant IFN-γ improves the outcome of infection [5]. Similarly, interleukin (IL)-18, also called IFN-γ–inducing factor, is involved

in defense against *Cn*. When mice are infected with a highly virulent strain of *Cn* via the pulmonary route, administration of IL-18 improves survival and reduces fungal burden in the lung and brain. Also, it reduces the level of *Cn* capsular glucuronoxylomannan (GXM) in the serum, another indicator of systemic cryptococcosis [6].

What proves the importance of the Th1 response in the lung is how it increases survival in those who receive

experimental treatments that activate it. A synthetic oligonucleotide containing an unmethylated CpG has proven beneficial against *Cn* in mice. This oligonucleotide is based on bacterial nucleotides shown to activate the immune response to several pathogens. CpG enhances the survival time and decreases the brain and lung fungal burdens of mice infected with *Cn*. The effect is via the Th1 response, as the Th1 cytokines IL-12 and IFN-γ increase in bronchiolar lavage fluid, whereas IL-4 decreases. Also, the activity is dependent on CD4+ T cells [7]. Thus, cell-mediated immunity, characterized by a Th1 response, is required for containing *Cn* in the lungs and ultimately preventing dissemination.

Antibodies are an important part of the immune response to *Cn* infection. Treating infected animals with antibodies to GXM can enhance survival and reduce lung fungal burdens and serum GXM [8]. In addition, human antibodies are effective against disseminated cryptococcosis. Transgenic mice expressing human antibodies can develop a protective antibody response to GXM mimetopes and acquire immunity to *Cn* [9•]. However, the role of antibody is complicated. B-cell–deficient mice have decreased survival time and increased lung fungal burdens compared with wild-type mice when infected with *Cn*. However, administration of antibodies against *Cn* does not increase the survival of these mice, even though it does in wild-type mice, indicating that passive antibody efficacy requires competent B cells. The B-cell–deficient mice have altered levels of inflammatory cytokines, suggesting alteration of the immune response. The decrease in survival time in these mice is therefore attributed to dysregulation of the immune response [10].

The role of antibodies in lung pathogenesis of *Cn* can be complicated by prozone-like effects. Some protective antibodies against *Cn* GXM are disease enhancing at high doses, and antibody efficacy varies with *Cn* inoculum. Antibodies that are protective with high inocula of *Cn* lose their protective ability or become disease enhancing at a lower inoculum. Thus, it seems that, at high doses, antibody may interfere with certain immune functions, such as oxidative killing by phagocytes. If this is the case, the high antibody dose might increase phagocytosis but inhibit killing, thereby promoting the dissemination of *Cn* [11]. The effect of antibodies is further complicated by the genetic background of the host. For example, IgG3 antibodies that are nonprotective in AJCr and C57BL/6J mice are protective in C57BL/6J × 29Sv mice. This may be due to the antibodies having different effects in the context of different cell-mediated immune factors [12]. Another example of antibody interaction with other immune components is its effect on the innate complement system. Different monoclonal antibodies to GXM recognizing various epitopes can alter the binding of C3 to the *Cn* capsule, which may ultimately effect opsonization of *Cn* and/or the inflammatory response [13].

An aspect of cryptococcosis not studied in the systems described above is long-term persistence of *Cn* in the lungs followed by later reactivation and dissemination. Evidence suggests that exposure to *Cn* is common [14]. The organism can remain latent in the body and later be reactivated by immunosuppressive therapies or disease. Rats provide the best available model for the study of latency and reactivation. Compared with mice, rats are less susceptible to cryptococcal disease. However, experimental infection with *Cn* shows that it can persist in the animals for more than 1 year, surviving in host cells inside the lungs. Treatment with the immunosuppressive agent dexamethasone leads to increased growth and dissemination to other organs [15]. Thus, the rodent model is useful for studying latent infection and reactivation, an important aspect of the pathogenesis of *Cn*.

The genetics and immune status of the host can greatly affect the dissemination of *Cn*. Certain host strains are more susceptible to disseminated *Cn* infections. Testing of several common laboratory murine strains for susceptibility to *Cn* shows that CBA/J mice are the most susceptible to infection and BALB/c the least, as determined by fungal burden in the brain. The difference is attributable to dissemination from the lungs, because no difference in fungal burden is seen after intravenous infection, only intratracheal infection. No single factor can explain the difference in dissemination between the host strains. Instead, a combination of factors, including antibody response, Th1/Th2 cytokine ratio, and the ability of *Cn* to replicate inside host macrophages, likely cause the difference in dissemination of *Cn* in these hosts [16].

Microbial factors

Numerous microbial virulence factors exist that promote microbial growth in the lung and exit into the bloodstream or lymphatic system, including capsule, laccase, phospholipase B, and inositol phosphosphingolipid-phospholipase. The polysaccharide capsule (Fig. 1) is one of the major virulence factors of *Cn* and as such, has numerous inhibitory effects on the host and the immune response. In addition, the capsule is thought to give the yeast a growth advantage in the lung because acapsular strains are generally cleared from the lung and rarely disseminate to the CNS [1].

The enzyme laccase catalyzes the first step of the pathway to produce melanin, another important virulence factor in *Cn* [2•]. When mice are infected intratracheally with either a laccase (-) or a laccase (+) strain, 70% of mice infected with the laccase (+) strain die by day 30 postinfection compared with 100% survival in mice infected with the laccase (-) strain [2•]. Interestingly, there is no difference in pulmonary clearance, leukocyte recruitment, inflammatory response, delayed-type hypersensitivity response, or cytokine production between the laccase (-) and (+) strains. The only difference is seen in mice infected with the laccase (+) strain where there are higher numbers of *Cn* in extrapulmonary sites, which suggests that laccase may be involved in dissemination. When the same strains are used to infect mice intrave-

nously, there are equal numbers of both strains growing in the brain and spleen after 1 week, suggesting that laccase is not involved in 1) survival and/or growth in extrapulmonary sites, 2) survival in the bloodstream en route to extrapulmonary sites, or 3) survival within the brain. Instead, laccase seems to play a role in escape from the lungs [2•].

Phospholipase B is a secreted enzyme that removes acyl chains from phospholipids, such as those found in cellular membranes and lung surfactant, and is an important *Cn* virulence factor [17]. When mice are infected intratracheally with H99 (wild type), a *plb* mutant, and the *PLB* reconstituted strain, there is significantly higher fungal burden in extrapulmonary sites (including the brain) of H99 and the *PLB* reconstituted strain than the *plb* mutant strain, suggesting a role for phospholipase in dissemination [18].

Because the phospholipids in the lung surfactant are preferred substrates of phospholipase B, the enzyme may initiate invasion of the lung interstitium and function in exit from the lungs [19]. When mice are infected intratracheally with H99, a *plb* mutant, and the *PLB* reconstituted strain, there is significantly less fungal burden associated with lung interstitial macrophages from the plb mutant at 24 hours, suggesting that phospholipase B facilitates the entry of *Cn* from the airways into the pulmonary interstitium. Also, infection with the *plb* mutant is confined to the lung, compared with mice infected with H99 or the *PLB* reconstituted strain, which show infection in blood monocytes, plasma, peritoneal macrophages, cerebral tissue, and hilar lymph nodes. These data suggest that phospholipase B is essential for hematogenous dissemination of *Cn* from the lung and for lymphatic spread from the lung to the regional lymph nodes [20•].

Inositol phosphosphingolipid-phospholipase C is involved in the degradation of inositol phosphoryl-ceramide to phosphorylinositol and phytoceramide. Because inositol phosphorylceramide is involved in intracellular and extracellular fungal growth [21], the degradation of this sphingolipid is important in the regulation of fungal growth and pathogenicity. When mice are infected intranasally with H99, an *isc* mutant, and the *ISC* reconstituted strain, there is significantly less fungal burden of the *isc* mutant compared with H99 and the *ISC* reconstituted strain in the brain, suggesting a role for inositol phosphosphingolipid-phospholipase C in dissemination. The *isc* mutant is only found extracellularly, is hyperencapsulated, and consequently induces very little inflammation compared with H99 and the *ISC* reconstituted strain. Interestingly, when immunocompromised mice (Tgε26) lacking T and natural killer cells are infected with the three strains, the *isc* mutant has no problem disseminating to the brain, which suggests that the host's immune response is important in preventing the *isc* mutant to disseminate. Indeed, when alveolar macrophages are depleted from mice infected with H99, the *isc* mutant,

and *ISC* reconstituted strain, the isc mutant disseminates easily. The authors then tested the ability of the strains to survive within activated macrophages, and not surprisingly, the *isc* mutant cannot survive after phagocytosis by an activated macrophage. Because the *isc* mutant is also extremely sensitive to acidic environments and oxidative and nitrogen stress, the authors conclude that inositol phosphosphingolipid-phospholipase C is important for allowing *Cn* to survive within macrophages in the lung [22].

Survival in Bloodstream or Lymphatic System
Host factors
Once *Cn* enters the circulatory system, it must survive for dissemination to occur. A potential mode of survival and growth is inside macrophages. Macrophages have a dual role in the pathogenesis of *Cn*. They can phagocytose and kill *Cn*, but this outcome does not always occur. Recent evidence suggests that the susceptibility of a host may depend on the ability of macrophages to contain *Cn* [23]. Alternately, *Cn* can survive ingestion and intracellularly replicate. Time-lapsed microscopy demonstrates that *Cn* replicates inside macrophages with a similar doubling time to fungal cells cultured in vitro. Intracellular growth of *Cn* is accompanied by accumulation of polysaccharide vesicles into the cytoplasm and eventual disruption of the phagolysosome. Fungal replication leads to eventual lysis and destruction of the macrophages and release of live *Cn* cells [24]. In addition, phagocytosed *Cn* can exit infected cells by phagosomal extrusion without lysis, allowing both the host cell and pathogen to survive [25••]. This phenomenon appears to require specific host and microbial factors, including the polysaccharide capsule [26]. Overall, these studies demonstrate that macrophages can be a vehicle for dissemination for *Cn* by providing a site for reproduction and perhaps a mechanism for distribution through the host.

Microbial factors
GXM is the major component of the capsule and significant amounts of polysaccharide accumulate in serum. GXM is believed to contribute to cryptococcal virulence through its many deleterious effects on immune function. This includes immune unresponsiveness, partly through the inhibition of T-cell activation and proliferation [27••,28], thus helping the organism escape killing while in the bloodstream.

Crossing the BBB
Host factors
For *Cn* to infect the CNS and cause meningoencephalitis, it must cross the BBB. One proposed mechanism is via infected phagocytic cells, also called the "Trojan Horse" mechanism. As discussed above, there is ample evidence indicating that *Cn* can use macrophages as a vehicle for

replication. Thus, it is possible that *Cn* "hides" in macrophages to cross the BBB, and then escapes such cells by lysis or extrusion.

Microbial factors

One way the fungus can cross the BBB is by transcellular penetration through brain microvascular endothelial cells (BMEC), or transcytosis. In vitro data provide compelling support for transcytosis. *Cn* cells can migrate across an artificial BBB in a few hours. Furthermore, when *Cn* cells are incubated with human BMECs in vitro, they penetrate the cells after microvilli engulf them. *Cn* can also be seen inside the BMECs, as well as exiting the opposite side of the cells. In vivo, *Cn* cells associate with brain endothelium within a few hours of infection [29••]. Thus, transcytosis is a likely mechanism for *Cn* to cross the BBB, and there is evidence that the polysaccharide capsule is involved [30].

Capsule changes resulting from phenotypic switching may help *Cn* cross the BBB. Phenotypic switching is defined as reversible colony morphology changes in the *Cn* capsule that arise in a population of cells at rates higher than the background mutation rate [31]. Switch variants show phenotypic differences in the morphology of the capsule, such as smooth, mucoid, and wrinkled, with the mucoid phenotype generally having increased virulence over the smooth phenotype [32]. Recently, phenotypic switching has also been described for *Cryptococcus gattii* (serotype B) where switching is involved in dissemination. When mice are infected intratracheally with either smooth or mucoid variants, only smooth variants are isolated from the brain. Interestingly, smooth variants also have smaller capsules than the mucoid variants. When mice are infected intravenously with either smooth or mucoid variants, mice infected with the smooth variant have significantly higher fungal burden in the brain, suggesting that the smooth variant with the smaller capsule size is better at crossing the BBB [33].

Mutations affecting capsule size can influence the ability of *Cn* to cross the BBB. Capsule size is important in crossing the BBB with an isc mutant where mutant cells are hyperencapsulated and cannot infect the CNS [22]. Additionally, strains deficient in galactose metabolism such that the capsule contains no galactoxylomannan are also hyperencapsulated and cannot colonize the brain after an intravenous infection in mice [34]. These data suggest that smaller capsulated cells may be more effective in blood-brain penetration and is consistent with an analysis showing a negative association between capsule size and virulence [35]. Thus, capsule size and changes in polysaccharide structure appear to be important for crossing the BBB [36•].

The metalloenzyme urease catalyzes the hydrolysis of urea to ammonia and carbamate and is an important virulence factor for *Cn* [37]. After intratracheal infection of mice with the wild type H99 strain and a urease deficient (-) strain, all mice infected with H99 die after week 6, whereas only 30% of the mice infected with the urease (-) strain die over the entire 8-week experiment. Additionally, the survivors have no visible evidence of *Cn* brain infection suggesting that deletion of urease prevents *Cn* infection/pathology in the brain and prolongs host survival [3•].

After intravenous, intratracheal, and intracerebral infection with H99, a urease (-) strain and the reconstituted urease (+) strain to determine where urease acts in dissemination, there is no difference in organ fungal burden between the strains on days 1 and 4. This suggests that urease is not involved in survival in the brain, lungs, spleen, or other extrapulmonary sites. When mice are infected intravenously, there is a 10-fold decrease in fungal burden in organs with closed capillary beds (brain, lungs, heart, and kidney), compared with organs with an open vasculature, such as the spleen, after 3 hours. This finding is interpreted to suggest that urease enhanced yeast sequestration within the microcapillaries of different organs. This finding may be related to dissemination to the brain because histologic analysis of the brain shows *Cn* within and in direct proximity of the microcapillaries, suggesting that H99 invades the brain via direct transfer across the BBB at the sites where *Cn* embolizes microcapillaries [3•].

Growth in the Brain
Host factors

Similar host defense mechanisms are needed to contain *Cn* in the CNS as are needed in the lungs. When *Cn* is infected intracerebral into mice, Th1 cytokines and chemokines are produced in brains of infected animals [38]. Furthermore, mice that produce a predominantly Th1 response (CBA/J) are more resistant than mice that produce a Th2 response (C57BL/6) after intracerebral infection [39].

Microbial factors

There are a few *Cn* factors that influence growth in the brain. The sterol regulatory element-binding protein (*SRE1*) plays a central role in adaptation to low-oxygen growth in *Schizosaccharomyces pombe* [40] and has recently been implicated in virulence of *Cn*. Interestingly, this mutant does not show a defect in the other steps of dissemination but is defective in its ability to establish infection and grow in the brain. Histologic data reveal that the *sre* mutant cells are limited to the outer regions of the brain (the leptomeninges and the cortex area) prompting the authors to conclude that sterol regulatory element-binding protein is required for cell growth in the neuronal parenchyma and the white matter regions of the brain as well as for fulminating CNS infection. Because the *sre* mutant is required for growth on nutrient-poor and low-iron media, the limited growth in the brain may be due to differences in available nutrients among the leptomeninges, the cortex, and the neuronal parenchyma [41].

Additionally, *SRE1* controls the expression of CTR4p, a high-affinity copper transporter that is also controlled by the copper-dependent transcription factor *CUF1*. When *CUF1* is knocked out, the *cuf1* mutant is avirulent in an intravenous model of infection in mice and has significantly less fungal burden in the brain. Using a CTR4-GFP construct, the authors show that CTR4 is upregulated in *Cn* in low copper conditions. Using CTR4 expression as an assay, they find that intracellular *Cn* (within J774.16 cells) and fungal cells isolated from the mouse brain have increased levels of CTR4, suggesting low copper availability. Interestingly, CTR4 expression from isolates of solid organ transplant patients with *Cn* infection is increased in patients with CNS infection compared with patients with a pulmonary infection. Thus, CTR4 expression (and low copper availability) is highly correlated with *Cn* growth in the brain [42•]. Interestingly, in the *sre1* mutant strain, CTR4 expression is upregulated, providing an additional explanation of low copper availability for poor growth in the brain [41].

Cryptococcosis and AIDS

Cn is an opportunistic pathogen. Normally, the host immune response is adequate to contain or eliminate the microbe. However, under certain conditions, *Cn* can escape and disseminate throughout the host. This involves a number of microbial and host factors that seem to be especially important in AIDS, as many aspects of HIV infection create a situation in which the host is susceptible. Thus, disseminated cryptococcosis is a very common disease in patients with AIDS, especially in the developing world where meningitis caused by *Cn* infection is found in up to 45% of patients with AIDS [43], mainly due to lack of access to highly active antiretroviral therapy (HAART) [44]. This is unfortunate, as histology studies have shown that AIDS patients given HAART have a better immune response against *Cn* infection and that patients without access to HAART offer little resistance to dissemination by *Cn* [45].

From the perspective of the host, HIV infection is associated with a reduction in CD4+ T cells, producing profound defects in cellular immunity that presumably markedly increase susceptibility and dissemination [46]. One defect is a shift toward a Th2 response that inhibits cellular immune responses that block dissemination [47], as seen in AIDS patients with disseminated *Cn* infection who have significantly higher levels of the Th2 cytokines TNF-α and IL-10 [48]. Additionally, HIV infection of alveolar macrophages impairs the patients' innate fungicidal activity against *Cn* [49]. Not only does AIDS increase susceptibility to cryptococcosis, but *Cn* may also influence the course of HIV infection because GXM has been shown to enhance the infectivity of HIV in susceptible cells, probably due to enhancement of the viral membrane protein gp120 binding to CD4 [50].

Conclusions

To understand the pathogenesis of invasive infection by *Cn*, it is imperative to discern how dissemination occurs. If we can understand exactly what factors contribute to each step of dissemination, it may be possible to develop new therapies for patients with cryptococcosis.

Acknowledgment

References and Recommended Reading

Papers of particular interest, published recently, have been highlighted as:
•   Of importance
••   Of major importance

1. Wilder JA, Olson GK, Chang YC, et al.: **Complementation of a capsule deficient Cryptococcus neoformans with CAP64 restores virulence in a murine lung infection.** *Am J Respir Cell Mol Biol* 2002, **26**:306–314.
2.• Noverr MC, Williamson PR, Fajardo RS, Huffnagle GB: **CNLAC1 is required for extrapulmonary dissemination of Cryptococcus neoformans but not pulmonary persistence.** *Infect Immun* 2004, **72**:1693–1699.
The first publication to dissect at which step of dissemination laccase is involved.
3.• 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.
The first publication showing how *Cn* uses urease to cross the BBB.
4. Huffnagle GB, Toews GB, Burdick MD, et al.: **Afferent phase production of TNF-alpha is required for the development of protective T cell immunity to Cryptococcus neoformans.** *J Immunol* 1996, **157**:4529–4536.
5. Kawakami K, Tohyama M, Teruya K, et al.: **Contribution of interferon-gamma in protecting mice during pulmonary and disseminated infection with Cryptococcus neoformans.** *FEMS Immunol Med Microbiol* 1996, **13**:123–130.
6. Kawakami K, Qureshi MH, Zhang T, et al.: **IL-18 protects mice against pulmonary and disseminated infection with Cryptococcus neoformans by inducing IFN-gamma production.** *J Immunol* 1997, **159**:5528–5534.
7. Miyagi K, Kawakami K, Kinjo Y, et al.: **CpG oligodeoxynucleotides promote the host protective response against infection with Cryptococcus neoformans through induction of interferon-gamma production by CD4+ T cells.** *Clin Exp Immunol* 2005, **140**:220–229.
8. Feldmesser M, Casadevall A: **Effect of serum IgG1 to Cryptococcus neoformans glucuronoxylomannan on murine pulmonary infection.** *J Immunol* 1997, **158**:790–799.
9.• Maitta RW, Datta K, Chang Q, et al.: **Protective and nonprotective human immunoglobulin M monoclonal antibodies to Cryptococcus neoformans glucuronoxylomannan manifest different specificities and gene use profiles.** *Infect Immun* 2004, **72**:4810–4818.
This paper shows that human antibodies to GXM are protective in mice.
10. Rivera J, Zaragoza O, Casadevall A: **Antibody-mediated protection against Cryptococcus neoformans pulmonary infection is dependent on B cells.** *Infect Immun* 2005, **73**:1141–1150.
11. Taborda CP, Rivera J, Zaragoza O, Casadevall A: **More is not necessarily better: prozone-like effects in passive immunization with IgG.** *J Immunol* 2003, **170**:3621–3630.

12.   Rivera J, Casadevall A: **Mouse genetic background is a major determinant of isotype-related differences for antibody-mediated protective efficacy against Cryptococcus neoformans.** *J Immunol* 2005, **174:**8017–8026.

13.   Kozel TR, deJong BC, Grinsell MM, et al.: **Characterization of anticapsular monoclonal antibodies that regulate activation of the complement system by the Cryptococcus neoformans capsule.** *Infect Immun* 1998, **66:**1538–1546.

14.   Goldman DL, Khine H, Abadi J, et al.: **Serologic evidence for Cryptococcus neoformans infection in early childhood.** *Pediatrics* 2001, **107:**E66.

15.   Goldman DL, Lee SC, Mednick AJ, et al.: **Persistent Cryptococcus neoformans pulmonary infection in the rat is associated with intracellular parasitism, decreased inducible nitric oxide synthase expression, and altered antibody responsiveness to cryptococcal polysaccharide.** *Infect Immun* 2000, **68:**832–838.

16.   Zaragoza O, Alvarez M, Telzak A, et al.: **The Relative Susceptibility of Mouse Strains to Pulmonary Cryptococcus neoformans Infection Is Associated with Pleiotropic Differences in the Immune Response.** *Infect Immun* 2007, **75:**2729–2739.

17.   Chen SC, Muller M, Zhou JZ, et al.: **Phospholipase activity in Cryptococcus neoformans: a new virulence factor?** *J Infect Dis* 1997, **175:**414–420.

18.   Noverr MC, Cox GM, Perfect JR, Huffnagle GB: **Role of PLB1 in pulmonary inflammation and cryptococcal eicosanoid production.** *Infect Immun* 2003, **71:**1538–1547.

19.   Santangelo RT, Nouri-Sorkhabi MH, Sorrell TC, et al.: **Biochemical and functional characterisation of secreted phospholipase activities from Cryptococcus neoformans in their naturally occurring state.** *J Med Microbiol* 1999, **48:**731–740.

20.•   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.
The first publication to describe how phospholipase is used by *Cn* in dissemination.

21.   Luberto C, Toffaletti DL, Wills EA, et al.: **Roles for inositol-phosphoryl ceramide synthase 1 (IPC1) in pathogenesis of C. neoformans.** *Genes Dev* 2001, **15:**201–212.

22.   Shea JM, Kechichian TB, Luberto C, Del Poeta M: **The cryptococcal enzyme inositol phosphosphingolipid-phospholipase C confers resistance to the antifungal effects of macrophages and promotes fungal dissemination to the central nervous system.** *Infect Immun* 2006, **74:**5977–5988.

23.   Shao X, Mednick A, Alvarez M, et al.: **An innate immune system cell is a major determinant of species-related susceptibility differences to fungal pneumonia.** *J Immunol* 2005, **175:**3244–3251.

24.   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.

25.••   Ma H, Croudace JE, Lammas DA, May RC: **Expulsion of live pathogenic yeast by macrophages.** *Curr Biol* 2006, **16:**2156–2160.
In concordance with Alvarez et al. [26], this paper demonstrates that *Cn* can replicate inside macrophages and subsequently be expelled without macrophage lysis.

26.   Alvarez M, Casadevall A: **Phagosome Extrusion and Host-Cell Survival after Cryptococcus neoformans Phagocytosis by Macrophages.** *Curr Biol* 2006, **16:**2161–2165.

27.••   Yauch LE, Lam JS, Levitz SM: **Direct Inhibition of T-Cell responses by the cryptococcus capsular polysaccharide glucuronoxylomannan.** *PLoS Pathog* 2006, **2:**e120.
This paper demonstrates that T-cell proliferation in response to various antigens is inhibited directly by GXM, a novel virulence mechanism.

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

29.••   Chang YC, Stins MF, McCaffery MJ, et al.: **Cryptococcal yeast cells invade the central nervous system via transcellular penetration of the blood-brain barrier.** *Infect Immun* 2004, **72:**4985–4995.
One of the first publications showing how *Cn* crosses the BBB.

30.   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.

31.   Guerrero A, Jain N, Goldman DL, Fries BC: **Phenotypic switching in Cryptococcus neoformans.** *Microbiology* 2006, **152:**3–9.

32.   Fries BC, Taborda CP, Serfass E, Casadevall A: **Phenotypic switching of Cryptococcus neoformans occurs in vivo and influences the outcome of infection.** *J Clin Invest* 2001, **108:**1639–1648.

33.   Jain N, Li L, McFadden DC, et al.: **Phenotypic switching in a Cryptococcus neoformans variety gattii strain is associated with changes in virulence and promotes dissemination to the central nervous system.** *Infect Immun* 2006, **74:**896–903.

34.   Moyrand F, Fontaine T, Janbon G: **Systematic capsule gene disruption reveals the central role of galactose metabolism on Cryptococcus neoformans virulence.** *Mol Microbiol* 2007, **64:**771–781.

35.   McClelland EE, Perrine WT, Potts WK, Casadevall A: **The relationship of virulence factor expression to evolved virulence in mouse-passaged Cryptococcus neoformans lines.** *Infect Immun* 2005, **73:**7047–7050.

36.•   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.
A paper that definitively shows that the capsule changes when crossing the BBB.

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

38.   Uicker WC, Doyle HA, McCracken JP, et al.: **Cytokine and chemokine expression in the central nervous system associated with protective cell-mediated immunity against Cryptococcus neoformans.** *Med Mycol* 2005, **43:**27–38.

39.   Huffnagle GB, McNeil LK: **Dissemination of C. neoformans to the central nervous system: role of chemokines, Th1 immunity and leukocyte recruitment.** *J Neurovirol* 1999, **5:**76–81.

40.   Hughes AL, Todd BL, Espenshade PJ: **SREBP pathway responds to sterols and functions as an oxygen sensor in fission yeast.** *Cell* 2005, **120:**831–842.

41.   Chang YC, Bien CM, Lee H, et al.: **Sre1p, a regulator of oxygen sensing and sterol homeostasis, is required for virulence in Cryptococcus neoformans.** *Mol Microbiol* 2007, **64:**614–629.

42.•   Waterman SR, Hacham M, Hu G, et al.: **Role of a CUF1/CTR4 copper regulatory axis in the virulence of Cryptococcus neoformans.** *J Clin Invest* 2007, **117:**794–802.
One of the first publications showing that nutrient accessibility is important for *Cn* to grow in the brain.

43.   Hakim JG, Gangaidzo IT, Heyderman RS, et al.: **Impact of HIV infection on meningitis in Harare, Zimbabwe: a prospective study of 406 predominantly adult patients.** *AIDS* 2000, **14:**1401–1407.

44.   Heyderman RS, Gangaidzo IT, Hakim JG, et al.: **Cryptococcal meningitis in human immunodeficiency virus-infected patients in Harare, Zimbabwe.** *Clin Infect Dis* 1998, **26:**284–289.

45.   Shibuya K, Hirata A, Omuta J, et al.: **Granuloma and cryptococcosis.** *J Infect Chemother* 2005, **11:**115–122.

46.   Huffnagle GB, Yates JL, Lipscomb MF: **T cell-mediated immunity in the lung: a Cryptococcus neoformans pulmonary infection model using SCID and athymic nude mice.** *Infect Immun* 1991, **59:**1423–1433.

47.   Cherniak R, Sundstrom JB: **Polysaccharide antigens of the capsule of Cryptococcus neoformans.** *Infect Immun* 1994, **62:**1507–1512.

48.   Lortholary O, Improvisi L, Rayhane N, et al.: **Cytokine profiles of AIDS patients are similar to those of mice with disseminated Cryptococcus neoformans infection.** *Infect Immun* 1999, **67:**6314–6320.

49.   Ieong MH, Reardon CC, Levitz SM, Kornfeld H: **Human immunodeficiency virus type 1 infection of alveolar macrophages impairs their innate fungicidal activity.** *Am J Respir Crit Care Med* 2000, **162:**966–970.

50.   Pettoello-Mantovani M, Casadevall A, Kollmann TR, et al.: **Enhancement of HIV-1 infection by the capsular polysaccharide of Cryptococcus neoformans.** *Lancet* 1992, **339:**21–23.