How Environmental Fungi Cause a Range of Clinical Outcomes in Susceptible Hosts

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Abstract

Environmental fungi are globally ubiquitous and human exposure is near universal. However, relatively few fungal species are capable of infecting humans, and among fungi, few exposure events lead to severe systemic infections. Systemic infections have mortality rates of up to 90%, cost the US healthcare system $7.2 billion annually, and are typically associated with immunocompromised patients. Despite this reputation, exposure to environmental fungi results in a range of outcomes, from asymptomatic latent infections to severe systemic infection. Here we discuss different exposure outcomes for five major fungal pathogens: *Aspergillus*, *Blastomyces*, *Coccidioides*, *Cryptococcus*, and *Histoplasma* species. These fungi include a mold, a budding yeast, and thermal dimorphic fungi. All of these species must adapt to dramatically changing environments over the course of disease. These dynamic environments include the human lung, which is the first exposure site for these organisms. Fungi must defend themselves against host immune cells while germinating and growing, which risks further exposing microbe-associated molecular patterns to the host. We discuss immune evasion strategies during early infection, from disruption of host immune cells to major changes in fungal cell morphology.

Introduction

Airway exposure to environmental fungi is constant, but very few are capable of establishing disease in humans. Of the ~120,000 documented fungal species and the estimated ~3 million species of fungi [1], only ~300 cause disease in humans [2]. Most of these 300 only rarely cause disease, while a dozen or so species cause the majority of fungal disease [3]. Consequences of exposure to environmental fungi vary from clearance by the host to establishment of a latent, reactivatable subclinical infection, to severe systemic disease [4–6]. Systemic fungal infections are a major cause of morbidity and mortality, killing >1.6 million people each year and costing the US healthcare system $7.2 billion dollars annually [7]. Moreover, mortality rates for systemic fungal infections are high. For instance, mortality rates can reach 80% for cryptococcosis [8] and 90% for aspergillosis [9] in resource-limited settings.

Life-threatening infections in immunocompetent individuals have been documented for all the fungi we discuss. However, the majority of life-threatening infections typically present in individuals with particular immunocompromising risk factors [4]. Thus, the outcome of infection is determined by a constellation of fungal traits and host susceptibilities (Table 1). In this review, we discuss host and fungal factors that contribute to infectivity, persistence within the host, and progression to life-threatening infections for five groups of fungi: *Cryptococcus*, *Aspergillus*, *Histoplasma*, *Blastomyces*, and *Coccidioides* spp. These common environmental fungi do not require an infectious life cycle for their survival. They are not transmissible between humans, nor are humans considered a particularly suitable niche for commensalism, such as it is for certain *Candida* spp. [10]. We do not discuss *Candida* spp., which are addressed elsewhere in this issue.

Aspergillus spp. and *Cryptococcus* spp. are considered some of the more cosmopolitan fungal

Table 1. Opportunistic fungi environments and risk factors for disease

Fungi	Natural niche	Severe clinical outcomes	Risk factors for life-threatening infections	Estimated life-threatening infections/year
Aspergillus	- Globally distributed	- IPA - Chronic pul monary aspergillosis - ABPA	- Neutropenia - Bone marrow transplants - Diabetes mellitus - Corticosteroids - COPD - Cystic fibrosis	>200,000 globally [4]
Cryptococcus	*C. neoformans* - Globally distributed - Found in soil and decaying trees - Associated with bird guano *C. gattii* - Distributed in tropical, subtropical and certain temperate climates - Associated with eucalyptus and bird guano	Meningoencephalitis	- HIV/AIDS - $CD4^+$ Lymphopenia - TNF inhibitors - Sarcoidosis - Renal failure - Chronic liver disease - Diabetes - Long-term steroid use - Solid Organ Transplants - Chemotherapy-induced immunosuppression - TNF-α inhibitors - Anti GM-CSF antibodies - Anti IFN-γ antibodies - Antibody deficiency - IgG2 deficiency - Fcγ receptor IIB variants - Mannose-binding lectin deficiencies - IL-12 receptor deficiency	>220,000 globally [8]
Histoplasma	- Ohio and Mississippi river valleys of North America - South and Central America - Sub-Saharan Africa - Humid or semi-humid river basins - Associated with bird or bat guano	Histoplasmosis	- HIV/AIDS - Organ/bone marrow transplants - TNF-α inhibitors - COPD - Smoking - Extreme young or old age - Histocompatibility complex haplotypes - IFN-γ receptor 1 deficiency	~25,000 in the United States [4]
Blastomyces	- Ohio and Mississippi river valleys - Great Lakes region - St. Lawrence river of Canada and North America - Some in Africa and India - Fresh water drainage basins - Forested areas near lakes and rivers	ARDS	- HIV/AIDS - TNF-α inhibitors - Solid organ transplants	~3,000 in the United States [4]
Coccidioides	- Southwest United States - Northern Mexico - Central Valley of California - Arid desert climates	Pneumonia (valley fever)	- HIV/AIDS - Solid organ transplants - TNF-α inhibitors - Extreme age - Diabetes - Pregnancy - African and Filipino ethnicities - IFN-γ receptor 1 gene loss of function or deletion (*IFNGR1* 818del14) - SNP in IL-12 Receptor β1 gene (IL12RB1 C186Y) - Gain of function mutations in STAT1	~25,000 in the United States [4]

Opportunistic fungi and risk factors for life-threatening infection. Opportunistic fungi with corresponding geographical range. Summary of host risk factors that contribute to severe disease and estimated amount of life-threatening infections [4,8]. All other references in text.

species. Due to their large geographical range, respiratory exposure to these fungi is commonplace [11]. *Aspergillus* spp. commonly cause both asymptomatic and symptomatic disease within the respiratory tract, but can also invade deeper tissues [5]. *Cryptococcus* spp. can establish latent, reactivatable infections within the lungs [12,13] and possibly the liver [14,15]. However, the presenting illness in those with impaired immune systems is usually central nervous system (CNS) infection and fungal meningoencephalitis [13]. *Histoplasma*, *Blastomyces,* and *Coccidioides* spp. belong to the Onygenales order of ascomycetes fungal pathogens, which also includes *Paracoccidoides*. These fungi are thermal dimorphs that undergo a reversible morphological transition from saprobic spore-forming hyphal growth at room temperature (25 °C) to parasitic yeast/spherule forms at core human body temperature (37 °C) [16]. Relative to *Aspergillus* and *Cryptococcus* spp., the thermal dimorphs tend to have more constrained environmental niches and geographical range. They are primary pathogens of humans, as they commonly infect immunocompetent people to produce self-limiting pneumonias [16]. However, certain immunocompromising risk factors greatly increase the potential severity of disease and extrapulmonary dissemination.

Ecology, Epidemiology, and Clinical Outcomes

Aspergillus

Aspergillus spp. are globally ubiquitous molds found in both the outdoor and indoor environments [17]. Approximately 90% of infections are caused by *Aspergillus fumigatus* [18], which is the focus of this review unless otherwise stated. Other disease-causing species include *A. flavus*, *A. niger*, and *A. terreus* [18,19].

Aspergillus spp. are globally distributed and possess a wide range of flexible traits that facilitate survival in diverse environments. These include heat tolerance [20], cold tolerance [21], drought tolerance [22], other stress tolerance [23–25], and the ability to utilize a variety of carbon and nitrogen sources [26,27]. While not all *Aspergillus* species, or even isolates within a species [24,28], possess the full range of these environmental survival traits, the range of phenotypes frequently correlates with virulence potential [24]. For example, *Aspergillus* spp. that are, on average, more likely to germinate at high temperatures are more likely to cause mammalian virulence [20]. The molecular mechanisms underlying these tolerances are of great interest to the medical mycology community, and we recommend that readers interested in these mechanisms start with the following reviews: [23,25,27,29,30].

Due to the global distribution of *Aspergillus* spp., preventing exposure is virtually impossible. Every time a human inhales, we are predicted to breathe in up to 10 fungal spores; this population frequently includes *Aspergillus* spp. [31]. Although *Aspergillus* spp. exposure can occur either indoors or outdoors [32], activities such as construction and building demolition near hospital sites further increase the risk of aspergillosis in vulnerable patients, presumably due to increasing airborne conidia levels when disturbing soil [33,34]. Municipal waste workers [35], others who work with compost material [35], and poultry farm workers [36] are at increased risk for *Aspergillis*-associated diseases.

Aspergillosis includes many forms. Invasive pulmonary aspergillosis (IPA) occurs when fungi enter the lung, invade and damage lung tissue, and enter blood vessels. Neutropenia [37] or neutrophil dysfunction [38] is the major risk factor for IPA. It will sometimes use the bloodstream to spread to distal sites, including the brain and skin [39]. Chronic pulmonary aspergillosis still damages lung tissue but progresses more slowly than IPA, does not invade blood vessels, and does not disseminate, although it causes extensive localized damage [40]. An aspergilloma is a localized fungal and inflammatory cell ball that grows in a pre-existing lung cavity [40] and is frequently asymptomatic [5,40]. Allergic bronchopulmonary aspergillosis (ABPA) is caused by hypersensitivity to *Aspergillus* antigens and increases asthma severity and airflow restriction in patients with asthma [41,42]. *Aspergillus* spp. can also colonize the lungs [43].

Aspergillosis is not necessarily progressive; patients with IPA do not always start with ABPA or even chronic colonization of the lungs [39], nor does colonization invariably lead to severe clinical outcomes [44]. This is particularly the case for neutropenic patients and those undergoing bone marrow transplant, the largest risk groups for IPA [19,39]. Mortality rates vary from 50% [45] to 95% [4] depending on the speed of diagnosis, patient condition, and treatment availability.

However, a number of conditions are associated with a positive *Aspergillus* spp. sputum sample, even without symptomatic disease. These include patients with diabetes mellitus and those administered corticosteroids [19]. Patients with pulmonary disorders are at greater risk for *Aspergillus* spp. colonization and infection. A study of patients in a pulmonary ward over a decade found that $<10\%$ of patient respiratory samples contained an *Aspergillus* spp., of which 45% displayed no further symptoms of aspergillosis [46]. All of the remaining 55% had a form of pulmonary aspergillosis, from IPA (13%) to ABPA or chronic necrotizing pulmonary aspergillosis (48%) [46]. Chronic obstructive pulmonary disease

(COPD) patients show an overall increase in IPA and *Aspergillus* colonization [47]. Recent work also established fungi in general and *Aspergillus* spp. in particular as part of the flora colonizing lungs of cystic fibrosis patients [48–52]. When *Aspergillus* colonization leads to ABPA, it exacerbates inflammation of cystic fibrosis patients' lungs and decreases in lung function [41,42].

Cryptococcus

Cryptococcosis is an invasive mycosis that primarily affects immunocompromised individuals, although it occasionally affects otherwise healthy individuals [13]. *Cryptococcus neoformans* and *Cryptococcus gattii* are basidiomycetes yeasts and the etiological agents of cryptococcosis, though most cases are attributed to *C. neoformans* [13,53,54]. *C. neoformans* is globally distributed in a multitude of environments, and exposure is near universal. In a New York City sample population for example, 70% of sera from children greater than 5 years old and 56% of sera from children 2 5 years old reacted with cryptococcal antigens [55]. Sera from children 1.1–2 years old had minimal activity against cryptococcal antigens [55].

Despite this high prevalence of exposure, individuals generally do not display clinical symptoms unless they are immunocompromised. Those with low CD4+ T-cell counts are at exceptionally high risk; cryptococcosis accounts for 15% of AIDS-related mortalities worldwide [8,13]. There are an estimated 220,000 cases each year with 180,000 deaths globally [8]. Although the advent of highly active antiretroviral therapy has helped reduce annual incidence of cryptococcosis globally, disease burden remains high in regions with limited access to highly active antiretroviral therapy, such as sub-Saharan Africa [8].

Cryptococcus was first identified in 1894 from a chronic granuloma of a tibial bone [53,54,56]. Since then more than 30 species of *Cryptococcus* have been identified, although two cause the majority of human disease: *C. neoformans* and *C. gattii* [13,56]. Each species has since been divided into two serotypes for further classification [13]. *C. neoformans* var. *grubii* (serotype A) accounts for 95% of cryptococcal cases, followed by *C. neoformans* var. *neoformans* (serotype D) and *C. gattii* serotypes B and C [13,56]. *C. neoformans* is often associated with decaying tree hollows and bird guano, particularly pigeons [13,53,54,56,57]. Although these birds are not symptomatically infected, they can harbor the fungus on their feathers and beaks and are likely an urban infection source [53,58].

C. gattii is not as widely distributed as *C. neoformans*. *C. gattii* is prevalent in tropical and semi-tropical regions of Australia and Southeast Asia [57,59], but can also be found in Hawaii, Brazil, and central Africa. *C. gattii* is frequently isolated from eucalyptus trees and koalas [13,57,60], which may serve as a reservoir in these areas. However, the Vancouver Island outbreak of the 1990s revealed *C. gattii* endemicity in more temperate climates, including the Pacific Northwest (North America) and parts of Europe [13,61–63]. Isolates from the Pacific Northwest also exhibited increased virulence when compared to more tropical *C. gattii* strains [59,64]. However, *C. neoformans* remains the predominant etiologic agent of disease, even in areas with endemic *C. gattii* [13,53,57].

Cryptococcal infections were rare prior to the AIDS pandemic, with fewer than 300 cases worldwide in the 1950s [54]. However, with increasing populations of immunocompromised individuals, *Cryptococcus* spp. have become a major threat. Upon immunological suppression, a latent or newly acquired infection spreads from the lungs to multiple body sites, including the prostate, skin, and eyes. Moreover, the fungus has a predilection for the CNS [65], and cryptococcosis more often presents as meningoencephalitis than pneumonia [13]. Although *C. neoformans* and *C. gattii* infections share a similar pathogenesis, *C. gattii* is more likely to cause symptomatic pulmonary infections and infections in individuals without obvious immunosuppression [59,66,67].

Cell-mediated immunity is critical for the clearance or control of *Cryptococcus*. A predominantly T helper-1 (Th1) reaction is essential for containing the infection, and when weakened may lead to fungal dissemination [13]. Due to a severe loss of CD4+ T cells, HIV/AIDS patients account for 80% of cryptococcal cases [8,13]. Other risk factors include $CD4^+$ lymphopenia, sarcoidosis, renal failure, chronic liver disease, diabetes, and long-term steroid use, tumor necrosis factor-α (TNF-α) inhibitors, solid-organ transplantation, and cancer chemotherapy-induced immunosuppression [13,68]. The cytokines interferon-γ (IFN-γ) and TNF-α are critical for controlling cryptococcal infection. Individuals with autoantibodies against IFN-γ and granulocyte-macrophage colony-stimulating factor (GM-CSF), which can decrease phagocyte and overall innate immune function, are also vulnerable [54,69–71]. In Australia, there is a higher rate of *C. neoformans* and *C. gattii* infection in indigenous populations, potentially due to a genetic and/or socioeconomic risk factor [59,69]. Smoking and old age were also risk factors for *C. gattii* infection during the Vancouver Outbreak [59].

Antibody deficiency may predispose otherwise immunocompetent individuals to *Cryptococcus* spp. Certain genetic polymorphisms and immune regulation deficiencies are linked to cryptococcal infections, including Fcγ receptor IIB variants [72,73], mannose-binding lectin deficiencies [74], IL-12 receptor deficiency [75], and IgG2 deficiency

[62,69]. Selective antibody deficiencies are hypothesized to render the host unable to mount a protective response to the cryptococcal capsule, a major antigen [59].

Histoplasma

Histoplasma spp., the etiological agents of histoplasmosis, commonly infect otherwise healthy and immunocompromised individuals in highly endemic areas [76]. Two varieties, *H. capsulatum* var *capsulatum* and *H. capsulatum* var *duboisii*, account for most human infections. Delayed-type hypersensitivity skin tests in regions of high endemicity suggest that ~90% of residents have been exposed at least once [77,78]. Most instances of exposure are either self-limiting asymptomatic or acute pneumonic infections exhibiting flu-like symptoms [78]. Only 1% will suffer severe or life-threatening illness and those patients are typically immunocompromised [78].

Although incomplete epidemiology and environmental sampling leaves the true range of endemicity unclear, *Histoplasma* spp. are now considered to span much of the globe [76,79]. It was initially thought that *H. capsulatum* was primarily constrained to the Ohio and Mississippi River Valleys of North America, with less defined endemicity in Central and South America [77,80,81]. A spike in histoplasmosis coinciding with the HIV/AIDS pandemic made it apparent that *Histoplasma* spp. had a wider geographical distribution than initially thought. Among the two of the major varieties, *H. capsulatum* var *capsulatum* is highly endemic in the soils of the Ohio and Mississippi River Valleys of North America, certain regions of South and Central America, and in sub-Saharan Africa [76,79]. *Histoplasma* spp. are also endemic in regions of Asia and Oceania, but the range is not clearly defined [82–84]. The geographical range for *H. capsulatum* var *duboisii* does not appear to extend beyond Africa, and overlaps with that of *H. capsulatum* var *capsulatum* in regions of endemicity [85,86].

In humid to semi-humid river basin habitats, *Histoplasma* spp. grow as a soil-resident spore-forming molds [79]. The haploid filamentous form of *Histoplasma* spp. also produces microconidia (2–5 μm) and tuberculate macroconidia (8–15 μm). Microconidia or sexually produced ascospores are likely the most common infectious propagules, as their small size allows them to reach terminal bronchioles.

Histoplasma spp. prefer soil rich in nitrogen, which is typically provided by bat or bird guano. Bats likely serve as a reservoir for *Histoplasma* spp. Bats are susceptible to pulmonary and disseminated *Histoplasma* infections and can shed the fungus in their guano [87,88], making caves and other bat roosts potential sources for human exposure [87,88]. Birds are resistant to infection, but natural and urban areas that commonly host high numbers of birds are also likely to be enriched with *Histoplasma* spp. due to high concentrations of guano. Thus, sites such as riparian habitats, chicken coops, and buildings amenable to roosting birds are also common sites of exposure [87]. Outdoor activities that disturb contaminated soil or guano, such as caving, excavation, and setting up campgrounds can increase the likelihood of inhaling spores [87].

The *Histoplasma* spp. are highly genotypically and phenotypically diverse, which results in a wide range of clinical presentations [89–93]. Although exposure and even self-limiting symptomatic infections are common in endemic regions, life-threatening infections are much rarer. The infecting *Histoplasma* strain, inoculum size, and host status all converge to produce a wide range of presentations including acute and chronic pulmonary infection, mediastinal lymphadenitis, and progressive disseminated infection [94]. Although *H. capsulatum* delays the onset of robust protective immunity through immunoevasive mechanisms discussed later in this review, development of a Th1 dominated response will typically control the infection [95–97].

Infections in immunocompetent individuals can produce asymptomatic to severe flu-like symptoms. In endemic regions, pulmonary nodules are common even in asymptomatic individuals, which can make diagnosis difficult [98]. Progressive dissemination can involve the liver, spleen, bone marrow, lymph nodes, gastrointestinal tract, and the CNS. However, dissemination is typically limited to patients with severe immunodeficiencies [94].

Cell-mediated immunity is critical for controlling *Histoplasma* spp. infection. As with many fungal infections, the most prevalent risk factor for life-threatening histoplasmosis is HIV/AIDS [99,100]. In hyper-endemic regions, up to 25% of HIV+ patients develop clinical histoplasmosis [101], with highly active antiretroviral therapy decreasing this rate [102]. Additional risk factors include organ/bone marrow transplantation [103,104], TNF-α inhibitors [105,106], COPD [102], smoking [76], extremely young or old age [94,104], and host genetics [107,108].

For solid organ or bone marrow transplant patients, most histoplasmosis diagnoses appear either within a year post-transplant or greater than 2 years post-transplant [103,104]. Patients receiving TNF-α blockers are at an increased risk for severe pulmonary histoplasmosis and progressive dissemination, as TNF-α is critical for activating macrophages and limiting intracellular proliferation [105,106]. COPD and smoking are both risk factors for chronic pulmonary histoplasmosis, especially in the elderly [76]. Infants (<2 years) and the elderly (>50 years) are at an increased risk due to natural immune deficits, especially when compounded with other risk factors [104]. Genetic risk factors

potentially include particular major histocompatibility complex haplotypes [107], and IFN-γ receptor 1 deficiency [108].

Blastomyces

The geographical range of the major causative agents of blastomycosis, *Blastomyces dermatitidis* and *Blastomyces gilchristii*, overlap with *H. capsulatum* in some regions of North America, though *Blastomyces* spp. likely extends further north [109]. *Blastomyces* spp. are prevalent in the Ohio and Mississippi River Valleys, the Great Lakes region, and the St. Lawrence River of the United States and Canada [110]. A newly identified species, *Blastomyces helicus*, appears to cause rare, pathologically distinct disease in the drier Western and mountainous regions of the United States and Canada [111]. Outside of North America, sporadically reported autochthonous cases occur in Africa and India [112,113].

In the environment, *Blastomyces* spp. mycelia penetrate soil and detritus, and produce conidia that are 2–10 μm in size. The ecological niches suitable for *Blastomyces* spp. are somewhat poorly defined, but in some ways appear similar to *Histoplasma* spp. *B. dermatitidis* and *B. gilchristii* reside in freshwater drainage basins, and can be commonly isolated in forested areas near lakes and rivers that contain acidic soil, large amounts of decaying vegetation, and bird guano [109,114,115]. Outdoor activities that disturb contaminated soil will aerosolize spores and increase the likelihood of exposure [110]. Unlike *Histoplasma*, *Blastomyces* is not known to infect bats or other animals as a means of propagation. However, symptomatic infection is particularly common in dogs and wild canids [116,117].

Within endemic regions, annual incidence of reported clinical *Blastomyces* spp. infection ranges from <1 human cases/100,000 people to >100 cases/100,000 people during outbreaks in the hyperendemic regions [118,119]. At least 50% of infections are mild or asymptomatic, and unlike *Histoplasma* spp., there is no reliable assay for prior exposure, so true incidence of infection is undetermined [115]. Blastomycosis commonly presents in immunocompetent patients, and up to 50% of exposures may result in symptomatic pneumonia [115,120]. However, severe clinical outcomes usually limited to immunocompromised patients. Most clinical cases (50%–75%) of blastomycosis are restricted to the lungs [119,121,122]. Pulmonary blastomycosis is symptomatically varied, typically presenting as acute or chronic pneumonia [109,123]. Severity ranges from mild and self-limiting pulmonary infections to acute respiratory distress syndrome (ARDS). ARDS is characterized by a rapidly overwhelming infection, pulmonary edema, and increased mortality [124–126]. *Blastomyces* spp. can also disseminate from the lungs to almost any organ, although it most frequently localizes to the skin [109,127,128]. Other common sites of dissemination include bone, the genitourinary system, and the CNS [109].

Risk factors for life-threatening infections include organ transplantation [129], HIV/AIDS [130], and TNF-α inhibitors [131,132]. Despite cell-mediated immunity being critical for resolving blastomycosis [133,134], the infection is relatively uncommon in patients with HIV/AIDS, especially compared to cryptococcosis or histoplasmosis [130]. However, HIV/AIDS increases the risk for ARDS and dissemination to the CNS [130]. Just as for histoplasmosis, protective immunity against blastomycosis is reliant on TNF-α [135], meaning TNF-α inhibitors can increase severity of disease [131,132].

Coccidioides

Coccidioides immitis and *Coccidioides posadasii* are the causative agents of coccidioidomycosis, otherwise known as Valley Fever. *Coccidioides* spp. are endemic in the arid deserts of the southwest United States and northern Mexico, with particularly high endemicity in southern Arizona, northwestern Mexico, and the Central Valley of California [136,137]. Sporadic cases and soil isolates discovered in the arid regions of eastern Washington and northeastern Utah suggest that the endemic range may be increasing or was greater than originally thought [138–140].

In the soil, *Coccidioides* spp. thrive in their mycelial form. Arthroconidia (spores) are the infectious propagule. They develop from within septate hyphae to form long chains that can dissociate when disturbed. *Coccidioides* can tolerate a wide range of soil pH and temperature, but appears to prefer alkaline sandy soils with high salinity [141–144]. However, arid desert soils are overall less nutrient rich than the river basins harboring *Histoplasma* and *Blastomyces* spp. Possibly due to this relative resource scarcity, the distribution of *Coccidioides* spp. can be patchy; presence of the fungus correlates with high salinity and plentiful essential nutrients such as iron and calcium [141,144].

Coccidioides spp. are also commonly isolated from animal middens, burrows, and carcasses [143,145,146]. Desert-dwelling animals such as rodents have been found with granulomatous *Coccidioides* spp. infections in their lungs [147], and *Coccidioides* spp. can resume hyphal growth in soil following the death of infected animals [148,149]. Genomic studies indicate that *Coccidioides* spp. evolution has been influenced through interactions with animal hosts, and that the fungus could utilize an expansive repertoire of proteases to access the organic matter from carcasses [150]. These observations suggest that *Coccidioides* spp. may exploit

animals and/or their burrows as reservoirs [143,145,146]. One caveat is that the most reliable way to isolate environmental *Coccidioides* spp. has traditionally been monitoring disease in laboratory mice inoculated with soil samples [137,151]. This method is cost-prohibitive and may bias sampling in favor of virulent isolates. A recently developed PCR-based method of detection should improve understanding of *Coccidioides* spp. ecology [152].

Coccidioides spp. soil growth, dispersal, and human infection tend to follow seasonal weather patterns in endemic areas. Milder temperatures allow for optimal mycelial growth [153,154]. Hot and dry periods facilitate maturation and dispersal of arthroconidia, particularly during strong winds [153,155–157]. Incidence of human infection often increases during dry seasons, and massive dust storms can carry infectious arthroconidia aloft for miles [153,155,156].

Large-scale delayed-type hypersensitivity skin testing beginning in the 1950s suggested that ~50% of residents in highly endemic areas had likely been infected [137,158,159]. Of those infected, 30%–50% were estimated to have developed symptomatic fungal pneumonia [137]. Similar to the other dimorphs, annual incidence of coccidioidomycosis is variable and dependent upon the frequency of activities that increase risk of exposure. There has been a recent uptick in coccidioidomycosis, especially in Arizona and California [137,160,161]. This increase in incidence could be due to changing weather patterns, influx of people from non-endemic areas, or urban expansion [137,161,162].

Most cases of coccidioidomycosis are self-limiting pneumonias with flu-like symptoms; dissemination occurs in less than 5% of immunocompetent patients [163,164]. Cell-mediated immunity is critical for resolving disease, and immunocompromising risk factors such as HIV/AIDS [165–168], organ transplantation [169–171], TNF-α inhibitors [131,172], extreme age [173], and certain genetic polymorphisms [174–176] can increase the severity of clinical outcome and frequency of extrapulmonary dissemination [177]. Although *Coccidioides* can disseminate to almost any organ, common sites include the CNS, skin, bones, and joints [178].

In contrast to cryptococcosis and histoplasmosis, HIV/AIDS does not seem to be as strong of a predictor for *Coccidioides* spp. dissemination to the brain [168,179]. Underlying genetic or hormonal factors appear to be more significant susceptibility markers for coccidioidal meningitis. Diabetes [180], pregnancy [181,182], and certain ethnicities (African and Filipino descent) [183,184] all correlate with increased risk for CNS infection [137,179]. Extrapulmonary dissemination occurs in ~30% of transplant recipients with coccidioidomycosis, making it a far more frequent occurrence than in the general population [169–171].

The potential genetic risk factors for susceptible populations are still unclear, but polymorphisms in genetic loci critical for proper Th1-mediated immunity have been associated with increased severity of clinical outcome. These include a loss of function deletion in the IFN-γ receptor 1 gene (*IFNGR1* 818del14) [174], and an SNP in the IL-12 receptor β1 gene (IL12RB1 C186Y) [175]. In addition, gain of function mutations in the IFN-γ signal transducer, STAT1, increased susceptibility to both *Coccidioides* and *Histoplasma* spp. [176]. The gain of function mutations increased IFN-γ expression, but decreased responsiveness to IFN-γ restimulation, suggesting that long-term aberrant regulation of Th1 responses impaired immunity to fungal infection [176].

Evidence for Latency

In addition to primary exposure immediately progressing to symptomatic disease in susceptible patients, there is clinical evidence in support of long-term persistence of environmentally acquired fungi within immunocompetent individuals. These "latent" infections can progress to symptomatic disease when individuals become immunocompromised. Much of the evidence in support of latent fungal infections comes from cases in which patients developed symptomatic disease years after migrating from endemic regions to nonendemic regions [185]. However, it is unclear how often symptomatic disease is the result of reactivated latent infections, or how the fungi are persisting in these cases. More research is needed to address these questions. For the purpose of this section, we will use "latent" to describe an asymptomatic infection capable of reactivating to symptomatic disease. We use "asymptomatic" to describe a subclinical infection that progresses in some cases, even if rarely. Use of the term "latent" does not, however, make any statement about whether the reactivatable microbe is metabolically dormant.

Aspergillus

Aspergillus spp., particularly *A. fumigatus*, can establish chronic infections in human lungs. There is not currently evidence that these infections are dormant and can be reactivated to cause invasive disease, as is the case for other fungal infections discussed below. Chronic *Aspergillus* spp. infections include aspergillomas and different types of chronic pulmonary aspergillosis [5,40]. The latter includes chronic cavitary pulmonary aspergillosis and chronic fibrosing pulmonary aspergillosis. Fungal-caused lung cavitations in chronic cavitary pulmonary aspergillosis can progress to chronic fibrosing pulmonary aspergillosis [5]. Antifungal treatment is necessary for chronic pulmonary aspergillosis [5,40]

but is not recommended for asymptomatic, non-fibrotic aspergillomas due to lack of efficacy [186–188]. Most patients with chronic pulmonary aspergillosis also have underlying lung conditions [5,189–191]. Aspergillomas are usually asymptomatic but occasionally progress hemoptysis and other forms of lung disease [192].

Aspergillus spp. colonization can also exacerbate inflammatory conditions. An estimated 4 million patients worldwide have ABPA [193], but diagnosis can take a decade or more [194]. Patients exhibit elevated IgE levels in response to *A. fumigatus* antigens [195,196]. ABPA is a particular problem for cystic fibrosis patients, in whom ABPA decreases respiratory function [42] and can cause lung fibrosis [41]. Approximately 9% of cystic fibrosis patients are diagnosed with APBA, whereas 1%–3% of asthma patients also have ABPA [197]. Not all cystic fibrosis patients with anti-*A. fumigatus* antigen IgE develop ABPA: genetic variants in HLA genes increase ABPA susceptibility in cystic fibrosis patients [198]. ABPA patients exhibit a Th2 cytokine response [197,199], a common trait among fungal infections that predicts worse patient outcomes [200,201].

Cryptococcus

Cryptococcus spp. cells can survive within pulmonary granulomas for years before reactivating to clinical disease under the right circumstances [11,185,202]. One study compared patient and environmental *C. neoformans* isolates from patients diagnosed with HIV/AIDS and cryptococcosis several years after immigrating to France from Africa [203]. They found that the strains isolated from the migrant patients were more similar to environmental isolates from Africa than Europe. This suggests that patients acquired *Cryptococcus* years prior to immunosuppression and diagnosis of fungal infection [203]. Autopsies have also revealed evidence of latent cryptococcal pulmonary nodules in patients who died of unrelated causes [204]. Furthermore, an association between increased sera reactivity against cryptococcal antigens and an increased risk for cryptococcal infections in transplant patients may indicate reactivated latent *Cryptococcus* as the source of infection. In a study that tested sera from transplant patients collected prior to transplant, patients who acquired cryptococcosis after transplantation showed more reactivity to cryptococcal proteins (52%) compared to approximately 9% reactivity among patients without cryptococcosis [205].

C. neoformans cells within granulomas may be able to achieve latent metabolic dormancy [206]. Long-term *C. neoformans* infection can be modeled in rats, where *C. neoformans* cells can survive for years in lung granulomas without clinical signs of illness and little to no cryptococcal cell growth in extrapulmonary organs [12]. While the metabolic state of these cells is unknown, immunosuppression with dexamethasone resulted in reactivation of fungal growth and extrapulmonary dissemination [12]. Additional work demonstrated that subclinical cryptococcal infection also worsened experimentally induced allergic inflammation within the lungs [207]. The ability of *C. gattii* to establish latent infections is less clear [59].

Histoplasma

There is some evidence that *Histoplasma* spp. can remain latent within granulomas for decades with potential for reactivation [208–211]. Reactivated histoplasmosis in transplant recipients from endogenous and donor-derived infections has also been documented [103,212,213]. Due to the high likelihood of repeat exposure in endemic regions, it is difficult to assess the frequency of latent infections. Evidence for latency is also derived from imported cases in non-endemic regions that occur years after the patient left an endemic area [208–211]. A new assay for *Histoplasma* latency assesses IFN-γ release by a patient's T lymphocytes when stimulated with *H. capsulatum* crude antigens; latently infected patients should exhibit increased IFN-γ [214].

Blastomyces

Blastomyces spp. can also remain latent for long periods within human lungs. The onset of primary blastomycosis can range dramatically from several weeks to months [215]. Delayed onset of clinical disease complicates pinpointing the source of outbreaks. In addition, reactivation of latent blastomycosis has been documented [130,216]. For example, in a set of HIV/AIDS patients, blastomycosis was diagnosed in patients who had not resided in endemic areas for several years [130]. The host and fungal responses that could lead to long-term latency are unknown but could involve a non-sterilizing granulomatous response following primary infection [185].

Coccidioides

A strong case for latent infection leading to reactivated coccidioidomycosis comes from transplant recipients. Up to 70% of cases in transplant recipients are diagnosed within one year of transplantation, and 50% occur within 3 months [169,217]. In addition, among a group of 13 patients who had acute coccidioidomycosis 1–4 years prior to transplantation, 11 developed coccidioidomycosis following transplantation [217]. Reactivated coccidioidomycosis can potentially occur decades after acute infection, as one patient developed disease 56 years after moving from California to non-endemic Spain [218].

Fungal Factors Promoting Infectivity and Virulence

Survival and evasion of early host immune responses in the lung are critical for establishing fungal infections and distinguish potential pathogens from other commonly inhaled but innocuous environmental fungi. Some of the strategies employed by environmental fungi are common across species, perhaps suggesting similar selective pressures in their native environments [219–222]. Others vary considerably. We provide an overview of early lung infectivity as a primer to readers new to the medical mycology field.

Fungi use many strategies to survive interactions with the host's immune system. Inhaled fungi face a largely inhospitable environment and must overcome a number of trials in order to survive and establish infection. The human airways are continually flushed by mucociliary clearance and invading fungal cells must initially be small enough to penetrate terminal bronchioles and alveoli where they might replicate [223]. Fundamental prerequisites for fungal infectivity include thermotolerance at 37 °C, and the capabilities for acquiring nutrients in the host environment, especially limiting nutrients sequestered by the host [30,224]. Metabolic adaptations to the host environment are extensive and are reviewed elsewhere [225]. During passage from the oral cavity to the lungs, conidia are exposed to airway epithelial defenses, and phagocytes deep in the lungs, particularly alveolar macrophages and neutrophils [226]. Fungi must survive attacks by phagocytes, germinate if the infectious propagule is a spore or conidium, and replicate in a nutrient-limiting, hypoxic environment. Many environmental fungal pathogens exhibit overlapping survival strategies to (1) grow within the host, (2) change their cell surfaces to survive host defenses, (3) evade and/or survive phagocytosis, and (4) manipulate host responses via secreted products.

Host-Associated Morphology and Growth

All of the environmental fungi highlighted here produce some form of small (usually <5 μm) cell as their primary infectious propagule. These propagules must evade or survive host immune recognition, in order to establish an infection. For instance, conidia are asexual spores that serve as the infectious propagules for *Aspergillus*, *Histoplasma*, *Blastomyces*, and *Coccidioides* spp. Conidia are frequently $\lesssim 5$ μm, which allows them to easily aerosolized and reach terminal bronchioles and alveoli. *Cryptococcus* does not produce asexual conidia; basidiospores (produced via either bisexual or unisexual mating [227]) and desiccated yeast cells are the most likely infectious propagules [11,13,53,228]. Once in the lungs, conidia germinate into vegetative fungal forms through extensive cell wall remodeling. These changes alter exposure of microbe-associated molecular patterns (MAMPs), triggering immune recognition.

Aspergillus

Aspergillus spp. conidia germinate as hyphae and if left unchecked can form large mycelial lesions in the lungs [40]. *Aspergillus* is obligately filamentous, and is the only fungal pathogen discussed here that undertakes hyphal growth in the host. Although *C. neoformans* occasionally produces pseudohyphae within host tissue, *Cryptococcus* spp. hyphal growth is generally restricted to mating and is associated with greatly reduced virulence [229]. Following germination, *Aspergillus* forms a complex network of hyphae known as a mycelium. The hyphae can penetrate lung tissue [230] and form aspergilloma, or "fungal balls," within the lung space. Aspergillomas' central cores consist of mycelia and extracellular polysaccharides [231], with immune cells and dead tissue on the periphery. They are initially asymptomatic but occasionally enter blood vessels and can cause hemorrhage [5,40]. Aspergillomas represent a usually asymptomatic form of infection that allows fungal persistence and can progress to severe disease [40].

Cryptococcus

Cryptococcus spp. grow predominantly as budding yeasts. The mating process has been extensively reviewed elsewhere [227,232], but sexual mating involves the fusion of two cells of opposite mating type (a and α), which undergo hyphal growth post-fusion. After nuclei fuse, basidia form at the ends of hyphae, where meiosis and spore formation occur [232]. Spores are thought to be the primary infectious propagule of *Cryptococcus* spp., are resistant to environmental stresses [233], and are virulent in mouse inhalation models of cryptococcosis [228,234]. *Cryptococcus* spp. can also undergo unisexual mating [235,236]. Since the α mating type predominates among *C. neoformans* isolates and the a mating type was thought extinct until 2003 [237], the existence of unisexual mating [235,238] provides a possible mechanism for formation of infectious propagules [227,239].

Within the host lungs, *C. neoformans* remains a budding yeast but displays enormous pleomorphism. In typical laboratory growth media, cryptococcal yeast cells are 5–7 μm in diameter [53]. A pulmonary infection, however, consists of cells ranging from 2–100 μm in size [240–242]. The differentiation of a subpopulation of cryptococcal cells into "titan cells" is particularly striking. Polyploid and ranging from 15 to 100 μm diameter, titan cells

have a thicker cell wall and a densely cross-linked capsule. Titan cells are resistant to phagocytosis and decrease phagocytosis of nearby smaller cells, which may allow them to promote long-term infection [243]. Environmental stimuli such as low nutrients, hypoxia, acidic pH, serum, and bacterial components can influence the generation of titan cells [244–246]. G-protein coupled receptor 5 and Ste3a are important for titan cell formation and signal through Gpa1 and the cAMP/PKA pathway [244–247]. This in turn activates the transcription factor Rim101, which is required for titan cell formation, and also regulates morphological transitions in *Candida albicans* [245,247,248]. Titan cells are rarely observed outside of the lungs, but can bud smaller haploid daughters which may be more likely to disseminate [53,240,249]. In addition, titan cells exhibit resistance to oxidative stresses [240], making them particularly suited for early infectivity.

Histoplasma

The "dimorphic" pathogens *Histoplasma*, *Blastomyces*, and *Coccidioides* spp. are so named because they have evolved morphogenetic programs coordinating the transition from environmental spore-forming molds to host-associated yeast/spherules. Virulence of these fungi is dependent on this transition, as yeast do not appear to be required for their environmental life-cycles [16]. Rather, the yeast-phase transition represents a specific program for pathogenesis [16,250–252]. The predominant environmental cue for all of these dimorphs is the shift from ambient (i.e., 25 °C) temperature to core human body temperature (37 °C). The import of exogenous cysteine is also necessary to initiate mitochondrial respiration and growth in recently transformed *Histoplasma* and *Blastomyces* yeast [253–255]. Elevated CO_2 tension (5% CO_2) in lung alveoli facilitates *Coccidioides* phase transition and enhances growth of *Histoplasma* yeast [160,256,257].

The genetic elements governing hyphal-to-yeast-phase transition by dimorphic fungal pathogens are best understood in *Histoplasma*. The transcription factors Ryp1, Ryp2, Ryp3, and Ryp4 [258–260] are all absolutely necessary for yeast form growth. The Ryp (*r*equired for *y*east-*p*hase growth) transcription factors form a positive feedback network to regulate themselves, the other three Ryp proteins, and broad yeast-phase transcription. Each Ryp protein is capable of associating with the *RYP1*, *RYP2*, and *RYP4*, promoters to induce transcription. The *RYP3* gene, although not directly bound by any of the Ryp proteins, still indirectly requires them for its expression [260]. Vea1 [261] and the histidine kinase Drk1 also positively regulate the transition to yeast [262]. Vea1 is required for the transition to yeast phase, but not its maintenance. The molecular mechanisms leading to initial upregulation of Ryp proteins and the potential links between the Ryp transcription factor network and Drk1 are unknown. Drk1 could facilitate signal transduction leading to *RYP* gene upregulation, but its phosphorylation targets have not been solidified [263].

Blastomyces

Blastomyces spp. yeast are multinucleate, and can be distinguished from *Histoplasma* spp. yeast under light microscopy by their broad-based bud neck and doubly refractile cell wall [255]. Despite these morphological differences, at least one of the factors governing yeast phase transition in *B. dermatitidis* overlaps with *H. capsulatum*. RNAi silencing of *DRK1* in *B. dermatitidis* completely prevents transition to yeast, and renders *B. dermatitidis* avirulent [262]. The *RYP1-4* genes governing yeast phase transition in *H. capsulatum* are also conserved in *B. dermatitidis*, but functional studies related to these genes in *Blastomyces* spp. have not yet been carried out [255,260].

Coccidioides

Coccidioides spp. arthroconidia differentiate into a host-specific form, but the morphology of *Coccidioides* spp. is dramatically different from *Histoplasma* and *Blastomyces* spp. yeast. Individual arthroconidia first adopt a more spherical form (spherules) via isometric growth. As they grow, spherules form an outer shell via synchronous internal division of their nuclei and cytoplasm, and the inner section fills with hundreds of individual (2-4 μm) endospores [160,264]. Mature spherules can measure 20–100 μm across. As with cryptococcal titan cells, mature spherules are too large to be engulfed by host phagocytes. When a spherule ruptures, it releases its endospores, which can then differentiate into new multi-endospore spherules. Lack of reliable methods for targeted genetic manipulation of *Coccidioides* has hampered the molecular understanding of the mechanisms mediating the arthroconidia to spherule transition. However, some insights have been made through comparative transcriptomic and proteomic studies performed on the mycelial and spherule forms [265–267]. For instance, *Coccidioides* spp. have homologs of *DRK1* and *RYP1–3* genes. None of these genes are significantly upregulated in *Coccidioides* spherules, but their contribution to phase transition is still unclear [265,266]. For instance, *DRK1* is also not overexpressed in *B. dermatitidis* yeast, despite Drk1 being necessary for yeast phase transition [262].

Cell Surface Remodeling

In addition to large-scale morphological changes, environmental fungal pathogens often alter their cell

wall composition or surface exposure during infection. Fungal cell walls contain many MAMPs, such as β-glucans and chitin. However, fungal cells often employ various mechanisms that mask inflammatory carbohydrates in the cell wall, allowing them to avoid or delay stimulation of the host immune system. For the fungi discussed here, this generally involves decorating the outer layer of the cell with less immunostimulatory molecules and/or reducing the content of immunostimulatory cell wall components.

Aspergillus

The outer surface of *A. fumigatus* conidia comprises hydrophobin protein rodlets over a layer of melanin. Beneath these layers, the conidial cell wall is rich in MAMPs, such as β-(1,3)-glucan and mannoproteins [268]. The germination rate is a major driver of immune cell detection, as germination induces changes in the fungal cell [269]. As conidia swell, MAMP exposure increases, rendering the fungi more easily detectable by host defenses. Swelling damages protective surface hydrophobins [270] and exposes β-(1,3)-glucan and other cell wall components [270–272]. β-(1,3)-glucan is detected by the dectin-1 receptor [273], which is essential for host defense against *A. fumigatus* [274]. Recognition of β-(1,3)-glucan activates signaling pathways which result in IL-8 upregulation [275]. IL-8 recruits neutrophils, which efficiently destroy *A. fumigatus* [275].

In contrast to conidia, *A. fumigatus* hyphae are coated in polysaccharides that facilitate infectivity and create a protective extracellular matrix. These secreted polysaccharides are predominantly galactomannan and galactosaminogalactan (GAG) [276]. GAG is synthesized within the cell, then cleaved by a plasma membrane-localized protein that is essential for infectivity and GAG production [277]. Extracellular matrix facilitates biofilm formation *in vitro* [276] and in the lungs [278], protecting mycelia from immune cells.

Cell surface changes are also tied to stress adaptation responses [279], which are in turn linked to nutritional adaptation to the lung environment [26,280,281]. The connections between pathways controlling different aspects of environmental responsiveness emphasize the importance of the fungi's ability to rapidly adjust to changing environments within the host.

Cryptococcus

Cryptococcus spp. cells rapidly expand their polysaccharide capsule *in vivo*, and capsule alone can more than double the size of a yeast cell [242,282]. Capsule composition is ~90% glucuronoxylomannan (GXM), ~10% galactoxylomannan, and ~1% various mannoproteins by mass [242,282]. Capsule is non-covalently attached to α-(1,3)-glucan in the cells wall, and deletion of the α-(1,3)-glucan synthase gene (*AGS1*) results in cells that still secrete capsule polysaccharide, but cannot maintain it on the cell surface [283]. Capsule production is stimulated by environmental cues that include low iron [284], host carbon dioxide levels [285], pH, nutrient availability [286], and additional stresses [242,282]. The transcription factor Rim101/PacC enables *C. neoformans* to sense extracellular pH and adapt its cell surface structure to evade immune stimulation [287–289]. Acapsular strains are avirulent in animal models and are rarely if ever observed clinically [290–292]. *C. neoformans* is also able to synthesize melanin and incorporate it into its cell wall [293]. Strains able to produce melanin showed less susceptibility to reactive oxygen species (ROS) than strains lacking melanin [294]. Melanin may also protect against microbicidal peptides such as defensins and can interfere with ingestion by phagocytic cells [294,295].

Histoplasma

Histoplasma spp. yeasts go to great lengths to shield cell wall β-glucans from the host. The cell walls of *H. capsulatum* yeast, but not mycelia, are highly decorated with α-glucan which cloaks underlying β-glucans from detection by the host pattern recognition receptor dectin-1 [296]. Remarkably, α-(1,3)-glucans are upregulated to such a degree in *H. capsulatum* yeast that during co-infection with *C. neoformans*, *H. capsulatum* can capture secreted cryptococcal GXM, providing further shielding from the immune system and heightened virulence [297]. RNAi-mediated suppression of *H. capsulatum*'s α-(1,3)-glucan synthase (*AGS1*) attenuates virulence in mice due to increased recognition of fungal cells and the resultant production of proinflammatory cytokines such as TNF-α [298,299]. Furthermore, *Histoplasma* yeast secrete the glucanases Eng1 and Exg8 to further reduce β-glucan exposure [300,301].

Interestingly, not all strains of *H. capsulatum* mask β-glucan with α-(1,3)-glucan [302]. *H. capsulatum* is divided into chemotype I and chemotype II strains based on cell wall characteristics. While chemotype II yeast cell walls contain high levels of α-(1,3)-glucan, chemotype I yeast poorly express *AGS1*, and lack apparent α-(1,3)-glucan in their cell wall during yeast-phase growth *in vitro*. In addition, when *AGS1* is completely inactivated in chemotype I cells, they maintain virulence [302,303] and can evade dectin-1 recognition [302]. Loss of Eng1 and Exg8 in both chemotypes increases dectin-1 recognition of β-glucan, indicating that β-glucanses in the chemotype I strains could at least partially compensate for the lack of α-(1,3)-glucan [300,301].

Blastomyces

Masking inflammatory carbohydrates in the cell wall may also help *Blastomyces* yeast prolong

immune evasion. Compared to hyphae, yeasts have less β-glucan and increased α-glucan content [304]. β-Glucans comprise ~40% of the hyphal cell wall, but only ~5% of the yeast cell wall [304]. This suggests a strategy similar to *Histoplasma*, in that yeast mask β-glucan to avoid immune stimulation through Dectin-1 or other host receptors [305].

Coccidioides

In *Coccidioides* spp., cell surface genes are among the most differentially expressed genes when comparing mycelial to spherule growth, highlighting their importance in phase transition and adaptation to the host environment [265,266]. *Coccidioides* spherules also upregulate transcription of the α-(1,3)-glucan synthesis gene *AGS1* [265,266], and dectin-1 recognition of *Coccidioides* contributes to host defense [306]. However, the relative content and exposure of α- and β-glucans in *Coccidioides* mycelia and spherules is unknown. *Coccidioides* chitin-related genes also display significant differential expression between the mycelial and spherule forms, including chitinase genes [265,266]. Inactivation of two chitinase genes (*CTS2* and *CTS3*) blocks endosporulation within maturing spherules and renders strains avirulent [307].

Interactions with Host Phagocytes

The human lung mucosa is under constant immunosurveillance, particularly by patrolling alveolar macrophages and dendritic cells. These phagocytes express an array of innate pattern recognition receptors and efficiently recognize and destroy invading fungi. They also recruit additional immune cells via cytokines and chemokines to control infection and provide a key link to adaptive immunity by presenting antigen in the draining lymph nodes. Fungal pathogen success during infection often involves limiting recognition by the innate immune system and surviving early interactions with phagocytes [226]. None of the fungal pathogens we discuss completely evade phagocytosis, and all of them have methods of surviving or subverting the microbicidal functions of the phagolysosome. In particular, *Cryptococcus* and *Histoplasma* spp. survive and even proliferate within macrophages to the extent that they are considered facultative intracellular pathogens.

Aspergillus

The initial host defenses against *Aspergillus* spp. conidia are airway epithelial and endothelial cells [308] and macrophages [309], but germinating conidia and hyphae are damaged or destroyed primarily by neutrophils [310]. Epithelial and endothelial cells endocytose conidia [308,311] and are then damaged by the conidia via a mechanism that requires conidia viability [311]. If conidia survive the airway and enter the lungs, they are frequently phagocytosed and destroyed by alveolar macrophages, particularly alternatively activated macrophages expressing Arg1 [312]. Alveolar macrophages efficiently kill conidia *in vitro*, though killing seems to be delayed until conidial swelling occurs [313]. Conidia can also prolong survival in phagocytes by manipulating phagolysosome maturation. For instance, conidial cell wall melanin inhibits phagolysosome acidification in macrophages and neutrophils [314,315].

Neutrophils are the primary defense against aspergillosis and neutropenia the major risk factor for invasive aspergillosis [37]. Neutrophils do not kill conidia but they do prevent conidial germination [316] regardless of opsonization [317]. Hyphae [318], particularly opsonized hyphae [317], are killed by neutrophils in a complement-dependent manner [319]. *A. fumigatus* will even form a new hyphal tip at a distal site following interactions with neutrophils, physical (e.g., barrier), or biological (e.g., removal of key nutrient) growth blocks to hyphal growth [320]. The secreted polysaccharide galactosaminogalactan partially protects hyphae from neutrophil by increasing resistance to neutrophil extracellular trap formation [321].

Cryptococcus

Alveolar macrophages interact with *C. neoformans cells* within 2 h of infection [322]. *Cryptococcus* spp. cells efficiently evade alveolar macrophages via their distinctive polysaccharide capsules. Acapsular strains are easily phagocytosed and cleared from the lungs [322], and the extracellular cryptococcal cells tend to exhibit larger capsule sizes than intracellular fungi [323]. In addition, capsular polysaccharide that detaches from the fungal cell accumulates within macrophages and dysregulates functions such as cytokine release and antigen presentation [324–326]. Not only do *Cryptococcus* spp. have methods of resisting phagocytosis, but they are also quite capable of operating as facultative intracellular pathogens. Studies in zebrafish show >50% of cryptococcal cells are internalized by phagocytes within 24 h of inoculation [323]. The fungus can replicate within the phagolysosome due to its ability to survive at low pH [327] and can subsequently escape from macrophages via lytic [326,328] and non-lytic mechanisms [329–332]. However, alveolar macrophages are capable of playing a protective role, as fungal burden in mice is enhanced when alveolar macrophages are depleted [333]. In addition, decreased phagocytosis reduces antigen processing and stimulation of T-cell proliferation [282,334,335].

Histoplasma

Histoplasma spp. takes their intracellular lifestyle one step further than *Cryptococcus* spp. *H. capsulatum* conidia and yeast are phagocytosed by alveolar macrophages within 10 min and readily proliferate within macrophage phagolysosomes, often leading to macrophage lysis [336–338]. The majority of yeast will remain intracellular over the course of infection [336–338], and the fungus can disseminate within macrophages to reach tissues such as the liver, spleen, bone marrow, and CNS [94]. Compared to macrophages, dendritic cells display better fungicidal and inflammatory activity against *H. capsulatum*, so proliferation within macrophages also provides a niche to escape further immune detection [339].

H. capsulatum facilitates macrophage adhesion and phagocytosis by localizing a small amount of the heat shock protein 60 (Hsp60), normally a cytoplasmic protein, to the cell surface. Hsp60 is recognized by macrophage CD18-family receptors such as complement receptor 3 (CR3) to induce phagocytosis [340]. Dendritic cells also express CD18-family receptors, but do not rely on them for phagocytosis of *Histoplasma*. As dendritic cells are much better at killing and processing *Histoplasma* yeast for antigen presentation [341], localization of Hsp60 to the cell surface may represent a targeted means for *Histoplasma* yeast to enter their macrophage safe havens [342]. Additional traits important for survival in phagolysosomes, such as acquisition of the limiting nutrients iron and zinc, are reviewed elsewhere [343,344].

Proliferating intracellular *H. capsulatum* yeast eventually lyse macrophages, after which they can spread to additional macrophages nearby. Macrophage lysis appears to be regulated at least in part by the yeast-specific calcium-binding protein 1 (*CBP1*) gene through the activation of host caspases [345,346]. *CBP1* is not necessary for maximal proliferation within macrophages *in vitro,* although disruption of *CBP1* does result in delayed proliferation and reduce lung fungal burden in mice [345]. This indicates that macrophage lysis is a critical step in perpetuating *Histoplasma* proliferation.

Blastomyces

Blastomyces spp. are not dedicated intracellular pathogens and often appear in extracellular spaces in histological samples [110,347,348]. There is also no current evidence *Blastomyces* spp. disseminate at a high frequency within macrophages. However, *Blastomyces* spp. still must overcome initial interactions with alveolar macrophages. Phagocytosis of *B. dermatitidis* conidia by alveolar macrophages begins within an hour of murine pulmonary infection [349]. Within 24 h, 30%–70% of *B. dermatitidis* cells appear to be intracellular, and can be predominantly found within alveolar macrophages [349,350]. Conidia are highly susceptible to killing by innate immune cells such as macrophages and neutrophils, while *B. dermatitidis* yeast cells tend to be more resistant due to their ability to suppress the production of toxic nitric oxide during alveolar macrophage infection [351–353].

Conidia that do survive phagocytosis can germinate into yeast cells and replicate within murine alveolar macrophages [349]. Depletion of alveolar macrophages and dendritic cells actually reduces early fungal proliferation of *B. dermatitidis* in the lungs, suggesting that macrophages might be an initial niche for *B. dermatitidis* germination into yeast and replication [349]. Alveolar macrophages are not completely permissive to *B. dermatitidis* yeast and can kill the fungus [354]. However, *B. dermatitidis* yeast within alveolar macrophages may be sheltered from more efficient killing by infiltrating neutrophils and monocyte-derived macrophages [352]. Previous exposure to *B. dermatitidis* enhances macrophage activity against the fungus [355], at least partially due to lymphocyte derived IFN-γ [356–358].

Coccidioides

Mature *Coccidioides* spp. spherules can reach 100 μm in size, which makes them too large to be engulfed by alveolar macrophages. However, inhaled arthroconidia and endospores released from ruptured spherules are certainly small enough to be phagocytosed. These smaller forms of *Coccidioides* spp. have subtler mechanisms that protect them from macrophage killing [348]. For example, *Coccidioides* spp. cells time the expression of an immunogenic cell surface protein to reduce opsonic phagocytosis. Mature spherules express SOWgp (spherule outer wall glycoprotein), which decorates the outer wall of spherules, and likely enhances adhesion to host tissues through interaction with various extracellular matrix proteins [265,359,360]. Loss of the *SOWgp* gene significantly attenuates virulence [359,360]. However, antibodies generated against SOWgp lead to increased phagocytosis and killing of *Coccidioides* [361]. To avoid SOWgp-mediated detection, small endospores released from mature spherules secrete the metalloprotease Mep1 in order to degrade SOWgp on the endospore surface [361].

In addition, alveolar macrophages that phagocytose *Coccidioides* spp. arthroconidia typically display poor fungicidal capabilities in the absence of help from lymphocytes [362,363]. *Coccidioides* spp. arthroconidia can also transition to immature spherules within macrophage phagosomes and appear to prevent phagosome-lysosome fusion [362,363]. Similar to *B. dermatitidis*, *Coccidioides* spp. can suppress toxic nitric oxide production by macrophages [364], which may partly explain why

pulmonary infection of mice with nitric oxide deficiencies has little effect on *Coccidioides* spp. pathogenesis [365,366].

Secreted Immunomodulatory Factors

A hallmark of plant and insect fungal pathogens is their expansive repertoire of secreted proteins, many of which are virulence factors that help shape a suitable microenvironment for fungal infection [367]. Although fungi capable of infecting mammals appear to harbor significantly fewer genes for secreted proteins, there are some notable examples [367]. Some of these secreted factors aid in counteracting the defense of the host's immune system, whereas others drive fungal adhesion and dissemination.

Aspergillus

A. fumigatus produces a number of factors that block host defenses and facilitate fungal survival. During the initial phagocytosis process, conidia are directed to the phagolysosome within macrophages, where they are destroyed by acidification and ROS. Fungal products such as melanin protect against ROS [368,369] and secreted catalases and oxydases block the formation and release of ROS by the host [370]. Fungal secreted proteases damage lung elastin and collagen I [371], which facilitate hyphal penetration of host tissue, fungal spread, and adhesion. This tissue damage also aids binding of conidia; when tissues are damaged, fibrin and fibronectin form at damaged sites. This damage recruits inflammatory cells and facilitates fungal adhesion [372]. Sialic acid on *A. fumigatus* conidia adheres to fibrinogen [373] and fibronectin [374], recruiting fungal cells to sites of tissue damage. The protein Aspf2 also binds host factors such as plasminogen, which is cleaved to plasmin and facilitates tissue invasion by damaging the lung extracellular matrix [375]. Aspf2 also binds factor H to conidial surfaces, which facilitates the cleavage of complement component 3b [376], downregulating the complement cascade.

Protein secretion by *Aspergillus* spp. is critical for infection. So many proteins are secreted that the unfolded protein responsepathway is induced, and an unfolded protein response signaling-deficient mutant is deficient in lung tissue growth [377]. Many of the tissue-damaging degradative enzymes secreted by *A. fumigatus* evolved to damage plant tissue but can also harm mammalian tissue [370]. Fungal elastases, which degrade elastin in lung alveoli [371], are common among clinical isolates and correlated with invasive disease [378,379], whereas strains without elastase activity are correlated with noninvasive, asymptomatic infections [379] or lack of pathogenicity [378].

Cryptococcus

Cryptococcus spp. secrete a multitude of enzymes that drive pathogenesis. Cryptococcal urease activity in the lungs promotes a non-protective type 2 immune response, and an accumulation of immature dendritic cells [380–382]. Urease also facilitates fungal transversal of the blood brain barrier [383]. Like *C. albicans* and *A. fumigatus*, *Cryptococcus* spp. secrete phospholipase B, which enhances virulence [384,385]. Phospholipase B activity enhances adhesion to respiratory epithelial cells [386], and is critical for survival within macrophages [387,388]. Phospholipase B appears to be attached to the cell wall [389], and is secreted via Sec14, a phosphatidylinositol transfer protein [390]. *C. neoformans* also secretes the metalloprotease Mpr1, which is required for CNS infection [391]. Mpr1 is sufficient for crossing *in vitro* blood brain barrier models. Expression of cryptococcal *MPR1* in *Saccharomyces cerevisiae* allowed the species to cross an *in vitro* blood brain model, when it is normally incapable of doing so [392,393]. Mpr1 may facilitate transversal of the blood-brain barrier by stimulating cytoskeletal reorganization through interactions with Annexin A2 [393]. Cryptococcal cells use this interaction in conjunction with the endothelial receptor EphA2 to successfully cross the blood brain barrier [394].

C. neoformans also secretes its primary cell surface polysaccharide GXM as a soluble exopolysaccharide (exo-GXM), which systemically suppresses inflammation [324]. Exo-GXM accumulates in the serum and infected tissues into the high μg/ml range [395,396], upregulating non-protective cytokines such as IL-10 and IL-4 while downregulating IL-2 [282,397]. Exo-GXM also inhibits T-cell proliferation *in vitro* [398], induces T-cell apoptosis during antigen presentation [399], and suppresses immune cell infiltration into sites of infection [400–402].

Histoplasma

H. capsulatum's intracellular lifestyle depends on secreted factors that are essential for survival and proliferation in an otherwise microbicidal environment. For instance, *H. capsulatum* yeast, but not mycelia, secrete superoxide dismutase Sod3 and catalase CatB to disable damaging ROS produced by macrophages [250,403,404]. Sod3 converts ROS to hydrogen peroxide, which is then degraded by CatB. Both yeast and mycelia produce an intracellular oxygen dismutase (*SOD1*) and catalase (*CATP*), neither of which are individually necessary for virulence [250,404]. CatB and CatP play redundant roles *in vivo*, as the *CATB*/*CATP* double mutant is attenuated for virulence in mice, while the single mutants maintain wild-type virulence [404].

Blastomyces

The yeast phase transition in *Blastomyces* spp. is also accompanied by an upregulation of secreted proteins, including transcription of superoxide dismutases (*SOD2* and *SOD3*) [405]. However, the most highly upregulated gene in yeast during pulmonary infection is the canonical virulence factor *BAD1* [405]. Bad1 is a secreted, multifunctional protein whose main functions are adhesion and immunosuppression. The C-terminus of extracellular Bad1 can bind cell wall chitin [406] and promotes adhesion to heparin sulfate on the host cell surface [407,408]. Fungal-bound and soluble Bad1 suppresses release of the critical cytokine TNF-α from macrophages and neutrophils by interacting with CR3 [135,409,410]. While fungal-bound Bad1 suppresses TNF-α through the induction of TGF-β, soluble Bad1 acts through a TGF-β-independent mechanism [409]. Bad1 can also suppress T-cell activation and secretion of host-protective cytokines IL-17 and IFN-γ [407]. Although complete loss of Bad1 renders *B. dermatitidis* essentially avirulent, the adhesive and immunosuppressive properties of Bad1 can be uncoupled. Deletion of just the C-terminal domain impairs adhesion but does not reduce the immunosuppressive properties of the soluble protein and has little effect on virulence in mice [406].

A more recently identified immunomodulatory virulence factor in *Blastomyces* is dipeptidyl-peptidase IVA (DPPIVA) [411]. This secreted protease is a molecular mimic of human CD26, a peptidase that degrades chemokines and has regulatory roles in hematopoiesis. *Blastomyces* DPPIVA cleaves GM-CSF to block maturation and activation of leukocytes [411]. RNAi-mediated silencing of DPPIVA greatly reduces fungal burden during murine lung infection [411].

Coccidioides

Compared to plant-associated members of the sister order Eurotiales, *Coccidioides* spp. genomes contain an expansion of proteolytic enzymes such as keratinases and extracellular serine proteases, which may benefit a life-style that is closely associated with both living animals and carcasses [150]. During pulmonary infection, *Coccidioides* spp. produce ammonia, which creates an alkaline microenvironment that contributes to host damage and fungal survival [347,412]. Urease, which is released when endosporulating spherules rupture, contributes to ammonia buildup and creating an alkaline microenvironment [412]. Urease catalyzes extracellular urea to produce ammonia, and the urease deficient mutant is hypovirulent in mice [412].

Discussion

Fungal diseases are often considered diseases of immunocompromised patients. Severe systemic fungal infections are typically associated with severe immunodeficiencies, particularly due to HIV/AIDS, or immunosuppressive therapeutics used for transplants and cancer chemotherapy. However, fungal infections can commonly result in milder clinical outcomes in those with stronger immune systems. Exposure to *Cryptococcus* spp. and the dimorphic fungi commonly result in latent infections lasting up to decades in immunocompetent hosts. The dimorphs also regularly cause symptomatic respiratory tract infections in otherwise healthy individuals. Whether a fungal infection remains subclinical or not depends primarily on the host condition; benign infections in the immunocompetent become systemic when patients become immunocompromised.

Why there are relatively few fungi that have evolved mammalian virulence remains largely speculative. Recently, the environmental lifestyle of these fungi has been appreciated with respect to human virulence. For instance, it is hypothesized that virulence in *Cryptococcus* spp., and potentially other environmental fungi, evolved through selection in the natural soil environment. *C. neoformans* interacts with a multitude of unicellular soil organisms, including predators such as amoebae and nematodes [219]. Amoebae can readily phagocytose *Cryptococcus* spp. [219], but phagocytosis is reduced by polysaccharide capsule induction, just as it is for macrophages [219,413]. Similar interactions with amoebae are observed for other fungi [249]. The tightly regulated thermal dimorphism of *Histoplasma* and *Blastomyces* spp. and their preference for guano-fertilized soil suggest that these fungi may have evolved alongside animals such as bats [414]. This relationship could have maintained a potential for virulence in other mammals, including humans. Finally, growing evidence suggests that *Coccidioides* spp. are associated with small desert mammals, which could serve as a reservoir or a selection for virulence attributes [415]. Despite these advances, our knowledge of their environmental lifestyles still severely lags behind our understanding of host-pathogen interactions. Further studies on the evolution of human disease-causing traits are critical for understanding the pathogenesis of environmental fungi.

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Abbreviations used:
CNS, central nervous system; IPA, invasive pulmonary aspergillosis; ABPA, allergic bronchopulmonary aspergillosis; COPD, chronic obstructive pulmonary disease; Th1, T helper-1; TNF-α, tumor necrosis factor-α; IFN-γ, interferon-γ; GM-CSF, granulocyte-macrophage colony-stimulating factor; ARDS, acute respiratory distress syndrome; MAMP, microbe-associated molecular pattern; GXM, glucuronoxylomannan; ROS, reactive oxygen species.

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