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REVIEW

Opportunistic yeast pathogens: reservoirs, virulence mechanisms, and therapeutic strategies

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Abstract Life-threatening invasive fungal infections are becoming increasingly common, at least in part due to the prevalence of medical interventions resulting in immunosuppression. Opportunistic fungal pathogens of humans exploit hosts that are immunocompromised, whether by immunosuppression or genetic predisposition, with infections originating from either commensal or environmental sources. Fungal pathogens are armed with an arsenal of traits that promote pathogenesis, including the ability to survive host physiological conditions and to switch between different morphological states. Despite the profound impact of fungal pathogens on human health worldwide, diagnostic strategies remain crude and treatment options are limited, with resistance to antifungal drugs on the rise. This review will focus on the global burden of fungal infections, the reservoirs of these pathogens, the traits of opportunistic yeast that lead to pathogenesis, host genetic susceptibilities, and the challenges that must be overcome to combat antifungal drug resistance and improve clinical outcome.

Keywords Opportunistic · Fungi · Yeast · Pathogen · *Candida* · *Cryptococcus* · *Histoplasma* · *Pneumocystis*

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Abbreviations

ABPA	Allergic bronchopulmonary aspergillosis
APECED	Autoimmune polyendocrinopathy–candidiasis–ectodermal dystrophy
cAMP	Cyclic AMP
CDC	Centers for Disease Control and Prevention
CGD	Chronic granulomatous disease
CLR	C-type lectin receptors
CMC	Chronic mucocutaneous candidiasis
CNS	Central nervous system
COPD	Chronic obstructive pulmonary disease
CRP	C-reactive protein
ECM	Extracellular matrix
GUT	Gastrointestinally induced transition
GXM	Glucuronoxylomannan
GXMGal	Glucuronoxylomannogalactan
HAART	Highly active antiretroviral therapies
HIES	Hyper-IgE syndrome
HSP	Heat shock protein
IFN-γ	Interferon-γ
IL	Interleukin
IRIS	Immune reconstitution inflammatory syndrome
MPO	Myeloperoxidase
MR	Mannose receptors
NF-κB	Nuclear factor-κB
NO	Nitric oxide
PAMP	Pathogen-associated molecular pattern
PCP	Pneumocystis pneumonia
PKA	Protein kinase A
PKC	Protein kinase C
PRR	Pattern recognition receptors
SAP	Secreted aspartyl proteinase
TGF-β	Transforming growth factor-β

TLR	Toll-like receptors
TNF-α	Tumor necrosis factor-α
Treg cell	Regulatory T cell
VVC	Vulvovaginal candidiasis

Introduction

The emergence of fungi occurred approximately 1.6 billion years ago [1, 2]. With an estimated 1.5 million species occupying a range of environments [3], fungal species are extraordinarily diverse. Fungi are a major cause of disease in insects, amphibians, plants and even other fungi, with incidences of infection reaching unprecedented levels in recent years. Strikingly, millions of acres of the world's forests have become victim to fungal infections. The blue stain fungus *Grosmannia clavigera* has devastated North American pine forests [4], and elm trees have been in significant decline since the twentieth century due to Dutch elm blight. Fungi also pose a major threat to food supplies, being responsible for the destruction of wheat and barley crops by wheat leaf rust and rice by rice blast fungus. Additionally, fungi pose a significant threat to animals, crippling bat and amphibian species worldwide [5].

Of the 1.5 million fungal species, around 300 are reported to be pathogenic in humans, and only a minority of these are common human pathogens [6]. Many fungi are commensal, forming part of our natural microbiota. Indeed, a recent study by Findley et al. [7] illustrated the diversity of fungal species on three different foot sites, and there is a growing appreciation that fungi have an important role in defining commensal microbial communities [8]. This commensal role allows fungi to infect humans in multiple ways. Ranging from cutaneous infections affecting 29 million North Americans each year [9], to superficial skin infections, to more than 2 million invasive fungal infections per year worldwide [10], commensal fungi have important roles in disease, being capable of switching to opportunistic pathogens. It is the opportunistic nature of fungal pathogens that triggered a stark rise in fungal infections in the late twentieth century, primarily in hosts with impaired immunity due to medical interventions such as chemotherapy for cancer, organ transplantation, or infection with HIV [11]. Overall, fungi are the cause of billions of infections worldwide, killing over 1.5 million people annually [10, 12]. Species of *Aspergillus*, *Candida*, *Cryptococcus* and *Pneumocystis* are at the forefront of fungal infections, accounting for approximately 90 % of human mortality cases [10]. Poor diagnostic tools and antifungal drug resistance accounts for a 50 % or higher mortality rate in patients with systemic fungal infections [11].

It has been postulated that global warming is in part responsible for the drastic increase in fungal infections, leading to the devastating loss of forests and crops worldwide. Remarkably, the common denominator of all human pathogenic fungi is the ability to grow at host temperatures [13, 14]. The steady rise in temperature upon climate change will selectively enable adaptation of fungi, broadening the array of species able to survive at host temperature [15]. Indeed, clinical isolates of the generally benign yeast *Saccharomyces cerevisiae* that exhibit enhanced capacity to grow at higher temperature (41 °C) compared to laboratory strains, were able to survive in mice [16]. The common fungal pathogens *Cryptococcus neoformans*, *Histoplasma capsulatum* and *Aspergillus fumigatus* are found in environments as diverse as pigeon excreta and soil, yet each retains the capacity to grow at 37 °C. Many of the genes required for growth at high temperatures in these pathogens are necessary for virulence and some are required for survival [17–19]. Consequently, high temperature growth is essential for pathogenesis. This review will focus on the reservoirs and mechanisms of pathogenic yeast infections, the challenges faced upon infection and the hurdles that must be overcome to combat antifungal drug resistance.

Global burden of fungal infections

Superficial and mucosal fungal infections are extremely common, but life-threatening systemic fungal infections are generally limited to immunocompromised individuals [20, 21]. The population of vulnerable individuals experiencing some form of altered immune function due to the HIV/AIDS epidemic, hospitalization, chronic illnesses, antibiotic-mediated microbiota alteration, or chemotherapies continues to increase [10, 22, 23], and has led to billions of cases of invasive fungal infections worldwide.

Cryptococcus

Cryptococcus neoformans is the leading cause of deaths due to fungal infections, with a global burden of nearly 1 million cases annually, and more than 620,000 deaths worldwide [23]. *Cryptococcus* is ubiquitous and globally distributed, with more than 70 % of children older than 5 demonstrating serum reactivity against cryptococcal proteins [24]. A major risk factor for cryptococcal infections is immunosuppression due to HIV/AIDS. In resource-limited areas with high rates of HIV/AIDS, the mortality rate approaches 70 % [23], and cryptococcal meningitis is considered an AIDS-defining illness [25]. Left untreated, cryptococcal meningitis is uniformly fatal.

In Western Europe and North America, the overall mortality rate of cryptococcal infections is 10 %, with approximately 700 deaths per year [23]. However, nearly 20 % of cryptococcosis cases in the US are in non-HIV patients [26], and the mortality rate of these infections can be greater than 30 % [27]. Many of these infections are in patients with conditions associated with immunosuppression or with solid organ transplants, although there are some incidences of underlying genetic susceptibility to fungal infections [26–28].

Another source of cryptococcosis in immunocompetent individuals is infections due to *Cryptococcus gattii*, which has caused an outbreak in the Pacific Northwest over the last 15 years [29–31]. The majority of these cases have been in immunocompetent individuals, and many cases were fatal despite antifungal therapies [31]. Although the origin of the outbreak was Vancouver Island, cases of *C. gattii* infections have been documented along the pacific coast and in Florida [32, 33].

An additional complication in cryptococcal treatment is the occurrence of immune reconstitution inflammatory syndrome (IRIS), where restoration of immune function after starting highly active antiretroviral therapies (HAART) results in enhanced and destructive immune responses to subclinical or cleared microbial infections [34]. Recent studies have reported that between 8 and 50 % of AIDS patients develop cryptococcal IRIS after treatment with HAART, and the mortality rate ranges between 30 and 60 % [35, 36].

Candida

Candida species are normal members of the human mucosal microbiota; studies have estimated between 25 and 40 % of people are colonized with *Candida albicans* at any given point [37, 38]. However, *C. albicans* also causes more than 400,000 deaths per year due to invasive candidiasis [39]. Disseminated *Candida* infections have an attributable mortality rate approaching 40 %, even with antifungal therapies [39].

Patients with HIV/AIDS or other forms of impaired immunity, especially neutropenia, are vulnerable to disseminated candidiasis [25, 39, 40]. Additionally, *Candida* species are the fourth most common cause of hospital-acquired bloodstream infections, and the incidence of *Candida* infections is increasing [41]. *Candida albicans* is the most common causal agent of nosocomial infection among the *Candida* species, followed by *C. glabrata*, *C. parapsilosis*, and *C. tropicalis* [41]. Many of these infections may be due to breakdown of mucosal surfaces during hospitalization or treatment, which then allows the superficial *Candida* colonization access to the bloodstream [42, 43]. Finally, *C. albicans* can form biofilms on medically implanted devices and catheters, creating a reservoir for bloodstream infections [44, 45].

Recently, mortality rates due to *C. albicans* infections in high-risk patients, such as stem cell transplant recipients or those undergoing therapy for leukemia, have decreased due to prophylactic fluconazole therapies [39, 42]. However, resistance can occur [46, 47], and the Centers for Disease Control and Prevention (CDC) has ranked fluconazole-resistant *Candida* as a serious threat [48].

Pneumocystis

Pneumocystis is an almost ubiquitous colonizer of human lungs, with approximately 80 % of children demonstrating serum antibodies to the organism [49–51]. However, it can also cause pneumocystis pneumonia (PCP) in immunocompromised hosts. In the early years of the AIDS epidemic, PCP was the most common clinical manifestation of decreased immune function [52]. Before HAART, approximately 65 % of AIDS patients in the United States exhibited PCP [52, 53]. Recent studies in developing nations have suggested that up to 40 % of HIV-infected patients exhibit PCP [52, 54]. An extrapolation of the current epidemiological reports suggests that there are more than 400,000 cases per year [10, 54].

Pneumocystis pneumonia remains one of the leading causes of morbidity and mortality worldwide [50]. In developed nations with readily available HAART, the mortality rate has dropped to approximately 10 % [55, 56]. However, in a recent study of pulmonary disease in AIDS patients in Uganda, the number of cases of PCP was lower than for tuberculosis or cryptococcal pneumonia, but the mortality was higher, with 75 % of the patients dying within 2 months of admission [57]. Additionally, *Pneumocystis* infections may also contribute to impaired lung function and increased mortality of patients with chronic obstructive pulmonary disease (COPD) [50, 58].

Aspergillus

Aspergillus is a ubiquitous filamentous fungus, and people are faced with continuous exposure to *Aspergillus* spores. As an opportunistic pathogen, *Aspergillus* mainly causes disease in patients with impaired immune function. Most infections are acquired exogenously instead of through reactivation of latent infections [59]. The most vulnerable population includes patients with neutropenia, organ or bone marrow transplant, or those undergoing immunosuppressive therapies [42, 59, 60]. Invasive aspergillosis is uniformly fatal if untreated, and even with current treatment options, mortality remains at 50 % [42, 61]. Current reports suggest that there are more than 200,000 cases of invasive aspergillosis per year [10].

Aspergillus can also cause allergic bronchopulmonary aspergillosis (ABPA), and recent estimates suggest that the global burden of ABPA is 4,837,000 patients [62, 63]. Patients with lung disease, such as COPD, cystic fibrosis, or emphysema are especially susceptible to ABPA [59, 60, 63]. Infection with *Aspergillus* can increase the mortality of these underlying diseases, contributing to the 100,000 deaths per year [10].

Histoplasma

Histoplasma capsulatum is endemic to the Midwest United States and Central America, though it has a global distribution [64]. *Histoplasma* is the most prevalent cause of systemic mycosis in Central America, and it infects several hundred thousand individuals annually [64, 65]. Recent studies estimate nearly 80 % of the adult population in the endemic areas show previous infections by *Histoplasma* [64]. Most of these infections occur in immunocompetent hosts, but the majority does not display symptoms. However, individuals who are exposed to large numbers of spores can suffer from acute pulmonary histoplasmosis. Additionally, the disease is more severe in patients with underlying lung disease [64, 65]. Disseminated histoplasmosis usually only occurs in individuals with compromised immune systems [64, 66]. Mortality due to disseminated histoplasmosis remains at 30 %, but it can be reduced with HAART [65].

Paracoccidioides

Paracoccidioides is an endemic dimorphic fungal pathogen in South America, and it is the most common cause of invasive mycosis in this region [65]. Nearly 10 million people are infected with this organism, but in most cases, the host immune system can prevent the conidia from causing disease [65]. However, individuals with compromised immune systems or those with decreased lung function, such as smokers, are vulnerable to expansion of the yeast from the lungs into disseminated disease [22, 65]. Interestingly, there is not a strong association of paracoccidiomycosis with HIV [67]. In some cases (between 5 and 13 %), patients experience relapse of paracoccidiomycosis due to reactivation of quiescent yeast cells [68].

Penicillium

Penicillium marneffei is an endemic fungal pathogen in Southeast Asia, causing disease primarily in immunocompromised patients, although infections in immunocompetent hosts also occur [69, 70]. In a study of AIDS patients in northern Thailand, it was the third most common cause of infection, after *Mycobacterium tuberculosis* and *C. neoformans* [71]. As an opportunistic pathogen, the prevalence of *P. marneffei* infections is increasing with the expansion of the HIV epidemic in Southeast Asia [72].

Penicillium marneffei infections are more common in the rainy season and are positively correlated with increased humidity [73, 74]. This suggests that disease occurs from primary infections shortly after exposure to the fungus [73]. These disseminated infections are uniformly fatal without antifungal treatments, but even with rapid administration of therapeutics, the mortality rate remains approximately 20 % [71, 74]. In children with immune deficiencies, the mortality rate is higher, at 55 % [70].

Coccidioides

Coccidiomycosis, also known as valley fever, is endemic to the Southwestern United States and Central America, and it causes thousands of hospitalizations and approximately ~400 deaths per year in the United States [75]. Coccidiomycosis is caused by *Coccidioides immitis* and *Coccidioides posadasii*, which occupy different geographic regions [76]. Although most infections are self-limiting lung infections, *Coccidioides* can also cause disseminated disease. Risk factors include compromised immune systems, but agricultural or construction work, outdoor activity, African or Asian ethnicity, increased age, or pregnancy also increase vulnerability to *Coccidioides*. Additionally, uncontrolled diabetes increases the risk of disseminated infections [77]. In patients with impaired T cell function and disseminated infection, mortality rates were 50 % [76].

Blastomyces

Blastomycosis is caused by infection with *Blastomyces dermatitidis*, a dimorphic fungal pathogen that is endemic in the Midwestern United States. Most infections are due to spore inhalation during outdoor activities, and approximately 30–50 % of infections are asymptomatic [78]. Of the symptomatic infections, most can be treated with antifungal drugs. However, mortality ranges between 4 and 22 % [78]. In a recent outbreak in Wisconsin, 70 % of the cases were hospitalized and 5 % died [79]. Only 25 % of the infected patients had any form of compromised immune system. However, there appears to be genetic risk factors for blastomycosis as the incidence rate is 12 times higher for Asians [79].

Rhizopus and Mucor

Mucormycosis is due to infections by basal fungi, most often by species of *Rhizopus* and *Mucor* [80]. Vulnerable

populations include patients with hematopoietic stem cell transplants or other hematological malignancies, those with diabetes, and those with compromised immune systems [80, 81]. The association between mucormycosis and diabetes is particularly strong; a recent study showed that 70 % of mucormycosis patients had diabetic ketoacidosis [81]. The mortality rate can be as high as 90 % for disseminated infections [82, 83]. Cutaneous mucormycosis can also occur after natural disasters, when environmental spores are disturbed and patients have open wounds [84]. The case-fatality rate for cutaneous mucormycosis can be as high as 80 % [84, 85].

Sporothrix

Sporotrichosis is usually caused by traumatic inoculation by material contaminated with *Sporothrix schenckii*, although there are some cases of inhalational sporotrichosis [86]. Although the infections are restricted to the site of infection, there are cases of disseminated sporotrichosis, mostly in immunocompromised individuals [67]. A recent study of cases in HIV-infected patients in Brazil found that the incidence of sporotrichosis was increasing to the point where there the incidence was comparable to histoplasmosis and cryptococcosis [67]. Of the patients with both HIV and sporotrichosis, almost 40 % were hospitalized for disseminated infection [67].

Trichosporon

Like *Candida*, *Trichosporon* can exist as a member of the normal human microbiota [87]. However, patients with malignant hematological disorders are vulnerable to infection with *Trichosporon* [87]. *Trichosporon* infections are the second most common cause of disseminated fungal infections in these patients, although the overall rate is low [88]. The mortality rate of patients with hematological disorders and *Trichosporon* remains at 11 %, even with antifungal therapies [88].

Reservoirs and nature of infection

Here we discuss the reservoirs and nature of infection, focusing on the predominant opportunistic yeast and yeast-like pathogens, *Cryptococcus*, *Candida*, *Histoplasma*, and *Pneumocystis*.

Cryptococcus

Cryptococcus species are found ubiquitously in nature, but only *C. neoformans* and *C. gattii* are considered serious human pathogens [89, 90]. The source of human infections are thought to be exclusively environmental, as there is no evidence of human to human transmission other than through contaminated medical devices [91]. Furthermore, concordance in phenotypic and genotypic characterization between environmental and clinical isolates within the same geographical region suggests they belong to the same fungal population [89, 92, 93].

Environmental isolates of these yeasts have been reported all around the globe, recovered from soil, dust, avian excreta, trees and other plants, domestic and wild animals, as well as marine mammals [94]. *Cryptococcus neoformans* is most often found in excreta from pigeons and other birds. In southern Africa, isolates are commonly found in the decayed hollows of the mopane tree [95]. *Cryptococcus gattii* is mostly isolated from tropical and subtropical regions in association with eucalyptus trees, as well as from soil, trees, and animals in the Pacific Northwest [94, 96]. Plant materials have also been shown to promote fertility and virulence of *C. gattii* [97].

Cryptococcal infection occurs upon inhalation of airborne fungal cells, generally spores or desiccated yeast [98]. Once inside the host lung, the yeast particles will encounter either alveolar macrophages or dendritic cells and trigger an immune response [99]. This can lead to clearance of the infection, or result in a localized asymptomatic latent infection that is contained within a granuloma, where the yeast is enveloped by immune cells [99, 100]. This latency period could last for years before reactivation of dormant infection occurs and disease symptoms develop [89, 101]. In the case of *C. neoformans*, reactivation occurs when host immunity is compromised, such as in patients with HIV/AIDS [89]. *Cryptococcus gattii* infections appear to be new events as they occur in both immunocompromised and immunocompetent individuals [102].

Active infection in the lung leads to pneumonia-like illness, with common symptoms including cough, fever, and dyspnea, among others [103, 104]. Fungal cells can subsequently disseminate from the lung through vascular or lymphatic systems to cause systemic infections, with the central nervous system (CNS) being the preferred destination [91, 100]. *Cryptococcus* can cross the blood–brain barrier either directly by transcytosis through the endothelial cells or with the aid of host monocytes [105–107]. Infection of the CNS and brain parenchyma is life threatening and results in cryptococcal meningitis and meningoencephalitis [104, 108]. Neurological symptoms include headaches, visual and hearing impairment, seizures, and mental status change [108, 109].

Candida

Although a large number of *Candida* species have been documented, only a few are known to cause disease in

humans [11, 110]. Over 90 % of all *Candida* infections are caused by five species: *C. albicans*, *Candida glabrata*, *Candida parapsilosis*, *Candida tropicalis*, and *Candida krusei*. Among these, *C. albicans* is by far the most prevalent, responsible for 90–100 % of superficial mucosal infections and 40–70 % of disseminated infections [11, 110].

Unlike most other pathogenic fungi, *Candida* species are a natural commensal of the human microbiome [111], found rarely in the soil and external environments, suggesting adaptation to a parasitic lifestyle [112]. The majority of *Candida* infections are from endogenous sources, derived from commensal populations acquired prior to disease development [11, 113]. Exogenous sources of infection are also common, especially in healthcare settings where transmission can occur from healthcare workers, other patients, and contaminated medical devices [11, 114].

Normally, *Candida* species exist harmlessly on our skin and mucosal surfaces as part of the commensal microbiota, colonizing the skin, oral cavity, gastrointestinal tract, and reproductive tract [111, 115]. However, disruption of the normal microbial flora or compromising the immune system may enable this fungus to overgrow, resulting in symptomatic infections. Due to the long evolutionary history with the human host, *C. albicans* is well adapted to survive and proliferate in the host environment. The switch from commensalism to pathogenesis involves significant changes in the fungus, including regulation of key virulence genes that allows it to quickly sense and adjust to different sites of infection in the body. *Candida* species must overcome different stresses, such as changes in temperature, pH, osmolarity, oxygen and nutrient availability [14, 112, 115–118].

Inflammations of the genitourinary tract are common clinical manifestations of *Candida* infections, which include vulvovaginal candidiasis (VVC) in women, balanitis and balanoposthitis in men, and candiduria in both sexes [119]. VVC, commonly referred to as thrush or yeast infection, typically presents as isolated occurrences of mild to moderate infection in otherwise healthy women, and are generally easily controlled with a single-dose therapy [110]. Episodes can occur either sporadically or due to predisposition resulting from a number of risk factors, including antibiotic use, pregnancy, diabetes, and immunosuppression [21, 120, 121]. A subgroup of patients will experience severe or recurrent VVC, in which case more rigorous and long-term antifungal therapies are required [110]. It is estimated that the majority of women develop at least one episode of VVC in their lifetime [122]. *Candida* balanitis occurs at a lower frequency than VVC, though the exact incidence is unclear due to the lack of population studies [119]. The disease is generally sexually transmitted and has been associated with diabetes and antibiotic use [123]. Candiduria, the presence of *Candida* species in the urinary tract, is commonly diagnosed in hospitalized patients, especially those with a urinary catheter [124–126]. Most cases of candiduria are asymptomatic or self-limiting, though it can cause increased vulnerability to bloodstream infections in high-risk patients, as well as increased mortality rates and hospital costs [119, 127].

Candida infections can also develop in the mouth or throat, resulting in oropharyngeal candidiasis. Clinical manifestations are typically characterized by white or red lesions on the surfaces of the tongue and oropharynx, which result in pain and burning sensations, alteration in taste, and tissue damage [128, 129]. Infection is generally associated with immunosuppression, antibiotic use, endocrine alterations, and denture use [128, 130]. It is estimated that over 90 % of HIV-infected patients will develop oropharyngeal candidiasis at some point during the progression of their illness [131].

Invasive candidiasis occurs when *Candida* species break the mucosal barrier, penetrating into deeper tissue and gaining access to the bloodstream [132]. Dissemination via the bloodstream allows the fungus to invade almost all body sites and organs, resulting in lethal systemic disease [110]. Major risk factors include immunosuppression, invasive medical procedures, and extended stay in the intensive care unit [11, 133, 134]. Early clinical symptoms of invasive candidiasis are non-specific and resemble other nosocomial infections, which impede accurate diagnosis and delays treatment, contributing to increased mortality [132, 135, 136].

Pneumocystis

Pneumocystis species are ubiquitous in nature, infecting a wide variety of mammalian hosts [11, 137]. While rodents were originally thought to be a natural reservoir, it is now known that *Pneumocystis* is not zoonotic. Current phenotypic and genetic evidence indicates that each mammalian species that contracts the disease has its own species of *Pneumocystis* [137–139]. *Pneumocystis jirovecii* is responsible for human infections. However, study of the organism has been hindered by the inability to culture *Pneumocystis* in vitro. Much of what we now know about *P. jirovecii* is based on direct clinical evidence or extrapolated from related animal models [50, 140].

PCR-based strategies have been used to type and track the spread of *P. jirovecii* [141, 142]. Children and immunocompromised individuals are identified as the most important sources of *P. jirovecii* infection [142–145]. While some environmental sources have been identified, transmission is thought to mostly occur through inhalation of airborne pathogens that spread from human to human [146, 147].

Pneumocystis has strong tropism for the lung, where it exists as an alveolar pathogen without invading the host [140]. Infections are typically asymptomatic or subclinical in the healthy host, but can develop into pneumocystic pneumonia in immunocompromised individuals, especially those with HIV infections [50]. Common clinical symptoms include progressive dyspnea, nonproductive cough, low-grade fever, and eventual respiratory failure with disease progression [140].

Histoplasma

Histoplasma capsulatum exists as a mold in soil environments and is often associated with bird and bat droppings, which enhance proliferation of the organism by accelerating sporulation [148]. Once contaminated, soil can yield the organism for years after the birds are gone [64]. Infections occur through inhalation of the aerosolized *H. capsulatum* from disturbed soils. Air currents can also carry the fungal spore for miles, resulting in infections far away from contaminated sites [148].

Most infections by *H. capsulatum* are asymptomatic in healthy individuals [148]. In children who were exposed for the first time and in individuals who experienced heavy exposure to the organism, pulmonary histoplasmosis may develop [64]. Common symptoms include fever, malaise, cough, and chest pain. Pulmonary infections can be complicated with infection of the mediastinal lymph nodes, resulting in Granulomatous mediastinitis.

In immunocompromised patients, such as those infected with HIV, disseminated histoplasmosis may occur [64, 149, 150]. Initial clinical presentation often includes fever, anorexia, and weight loss. Subsequent severe disease manifestation includes sepsis, as well as failure of respiratory, renal, and multi-organ systems.

Mechanisms of pathogenesis

Pathogenicity is the ability of a microbe to cause damage to the host [151]. The ability of an opportunistic pathogen to cause disease requires the expression of virulence factors. Here, we outline some of the traits that enable pathogenesis of the major opportunistic yeast pathogens of humans, *C. albicans*, *C. neoformans*, and *H. capsulatum.*

High temperature growth

Fungi must be capable of surviving in human host conditions. One trait absolutely required for pathogenicity in humans is the ability to grow at human body temperature, 37 °C [14]. The most prevalent fungal pathogen, *C. albicans*, is a natural commensal of mammals and is thus intrinsically competent for growth at 37 °C. Despite this thermal adaptation, the heat shock response has been retained in *C. albicans* and influences virulence [152], presumably reflecting the importance of febrile temperatures during systemic infection [153]. In *C. albicans,* the heat shock response is governed by the transcription factor Hsf1, and the molecular chaperones Hsp70 and Hsp90 [14]. Mutant *C. albicans* strains containing inactive Hsf1 are thermosensitive and display reduced virulence in a murine model of systemic candidiasis [154]. Furthermore, the membrane dynamics of *C. albicans* are crucial for sensing changes in temperature, with depletion of *OLE1*, encoding a fatty acid desaturase, impairing activation of Hsf1 [155]. Temperature is a central cue that enables the morphogenetic transition of *C. albicans* from yeast to filamentous growth through complex cellular circuitry [156].

Although the saprobe *C. neoformans* is usually found in environmental locales, the ability to grow at human physiological temperatures allows it to cause disease. Several signaling pathways have been identified as essential for *C. neoformans* growth at elevated temperature, including Ras1 and Cdc42 signaling [157], the unfolded protein response [158], the histone acetyltransferase Gcn5 [159], the cell wall integrity pathway [160], and calcineurin signaling [19]. In addition, increased expression of *C. neoformans* Hsp90 and other heat-shock proteins is observed upon infection of a murine lung [161], suggesting a role for Hsp90 in adapting to host conditions. Mutants that are unable to grow at 37 °C are unable to cause disease in murine models of cryptococcosis.

Like *C. neoformans*, the dimorphic fungus *H. capsulatum* can be found in the soil. However, *H. capsulatum* forms mycelia in the environment and changes to the yeast form upon inhalation by a mammalian host. The increase in temperature to 37 °C is key for this transition, and membrane dynamics may be involved in sensing this cue [162]. For example, addition of saturated fatty acids with a concurrent increase in temperature amplifies the *H. capsulatum* heat shock response [163].

Adaptation to pH

Opportunistic fungal pathogens must not only adapt to variations in temperature, but also pH. In the host, the pH can range from acidic (in the vaginal and gastrointestinal tracts and the immune cell phagolysosome) to basic (such as in the blood or saliva) [164]. Therefore, pH is a powerful signal for entry into the host bloodstream or other microenvironments. Additionally, environmental pH can lead to alterations in nutrient bioavailability.

In *C. albicans*, alkaline conditions result in cleavage and activation of the transcription factor Rim101, which leads

to the upregulation of genes required for growth in alkaline conditions, including genes involved in iron acquisition and morphogenesis [165]. Additionally, Rim101 regulates expression of genes with roles in adhesion and modulating the fungal cell wall [166]. *PHR1* and *PHR2* are pH-responsive genes regulated by Rim101, with *PHR2* being required for virulence at acidic infection sites (such as the vaginal tract), whereas *PHR1* is required at alkaline sites of infection (such as the bloodstream) [167]. Mutants lacking Rim101 have reduced virulence in a mouse model of systemic candidiasis [168].

Cryptococcus neoformans also uses the Rim101 transcription factor to adapt to varying environmental cues. Similar to *C. albicans*, *Cn*Rim101 is required for growth in neutral/alkaline conditions, and also regulates expression of genes required for iron and metal homeostasis [169]. However, in *C. neoformans*, Rim101 is also activated by the cyclic AMP (cAMP)/protein kinase A (PKA) pathway [169]. Rim101 is required to modulate many important *C. neoformans* virulence traits, such as attachment of the polysaccharide capsule [169], the morphological change to titan cells [170], and modulation of the host immune system [171, 172]. Upon phagocytosis by macrophages, *C. neoformans* enters into an acidic phagolysosome; as a facultative intracellular pathogen, *C. neoformans* is well adapted for growth in acidic conditions [173].

Nutrient limitation

Key to survival is growth. Organisms are often nutrient starved within the host and must adapt to changes in available host nutrients. One mechanism by which *C. albicans* can respond to limited glucose is by secreting ammonia and autoalkalizing its surroundings to induce filamentation [174]. For *C. neoformans,* phagocytosis by macrophages induces a starvation stress response that includes upregulation of amino acid and sugar transporters, gluconeogenesis and fatty acid metabolism [175].

As iron levels are tightly controlled within the host, microbes must have mechanisms to acquire this essential cofactor [176]. Although *C. albicans* and *C. neoformans* do not appear to produce their own siderophores, which are high-affinity iron-binding proteins, they are capable of transporting xenosiderophores produced by other organisms [177, 178]. Additionally, both *C. neoformans* and *C. albicans* are able to use iron from hemoglobin and ferritin as iron sources [179, 180]. *Cryptococcus neoformans* utilizes a ferric reductase system involving the iron permease Cft1 and ferroxidase Cfo1 to acquire iron from host transferrin [181, 182]. Mutants lacking these enzymes show a reduced virulence in a mouse model of cryptococcosis. Iron levels are sensed by a master transcription factor, Cir1, which directs the expression of genes for iron acquisition. Cir1 is also involved in regulating growth at physiological temperature, capsule formation, and melanin production [183], highlighting the central role of iron regulation in *C. neoformans* virulence.

Candida albicans is able to acquire iron from hemoglobin by first binding hemoglobin to a receptor on the cell wall and subsequently endocytosing this complex [184]. The heme oxygenase Hmx1 then releases ferrous iron [185], carbon monoxide, and biliverdin [186]. As carbon monoxide has immunosupressive properties [187], its production by Hmx1 may decrease the ability of the host immune system to clear a *C. albicans* infection. The *hmx1* mutant is unable to cause disease as this mutant is unable to grow in iron-limited conditions and has a defect in carbon monoxide production [186, 188]. Additionally, the transcriptional activator Sef1 induces iron-uptake genes and enables virulence, whereas the transcriptional repressor Sfu1 diminishes expression of iron-uptake genes and enables gastrointestinal commensalism [116]. These genetic programs may be fundamental to enabling *C. albicans* to survive iron depletion in the bloodstream while minimizing iron toxicity in the gut.

Histoplasma capsulatum is able to both produce siderophores and utilize reductive iron assimilation to obtain the iron necessary for growth [189]. *Histoplasma capsulatum* produces hydroxyamate-type siderophores, and these siderophores are required for proliferation within macrophages and infection in mice [189, 190]. Production of siderophores is negatively regulated by the GATA-type transcription factor *SRE1* [191]. *Histoplasma capsulatum* can also take up ferrioxamine B, a xenosiderophore, via ferric reductases [192].

Changes in cellular shape and size

Host physiological temperature induces a filament-to-yeast transition in dimorphic fungi, such as *H. capsulatum* (Fig. 1). This switch from mycelia to yeast growth at host temperatures is a requirement for *H. capsulatum* virulence, and the histidine kinase Drk1 is a key regulator of this transition [193]. *DRK1*-silenced strains show a drastic reduction in virulence in a mouse model of histoplasmosis [193]. In addition, Drk1 regulates expression of two important *H. capsulatum* virulence genes, *CBP1* and *AGS1* [193]. The DNA-binding protein Ryp1 is also essential for yeast growth, and is related to the *C. albicans* master transcriptional regulator of phenotypic switching, Wor1. Ryp1 is a master regulator of morphogenesis, as it is required for the expression of yeast-phase-specific virulence genes and repression of mycelial-specific genes [194]. Recently, a temperature-responsive regulatory circuit composed of four Ryp proteins was identified that controls both the transition from filamentous to yeast forms and the

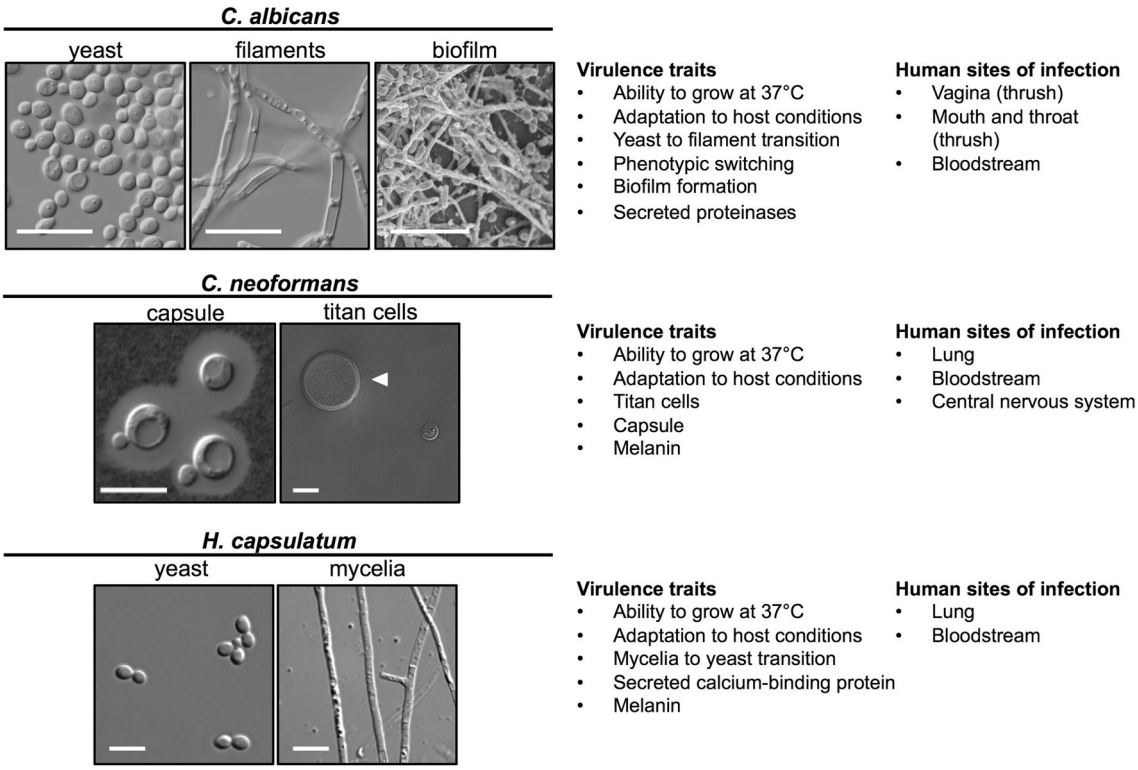

Fig. 1 Yeast virulence traits. *Top*, *C. albicans* can exist as yeast, filaments, or as a biofilm. Wild-type yeast cells were grown in rich medium at 30 °C for 24 h. Filaments were grown in RPMI at 37 °C for 24 h. The biofilm is fluconazole treated and was formed in a rat central venous catheter. *Scale bar* is 20 μm. *Middle*, *C. neoformans* cells stained with India ink to highlight the capsule. *Arrow* indicates the size of a titan cell as compared to a normal cell. *Scale bar* 10 μm. *Bottom*, *H. capsulatum* can exist as either yeast or as mycelia. *Scale bar* is 5 μm

expression of virulence genes such as *YPS3* and *CBP1* [195].

Strikingly, host physiological temperature induces the opposite morphological transition in the commensal fungus, *C. albicans*, from yeast to filament compared to the filament-to-yeast transition initiated in dimorphic fungi (Fig. 1). *Candida albicans* is able to undergo various morphological transitions that facilitate its ability to colonize and infect different niches within the human host. The most striking of these morphological transitions is that from yeast to filamentous forms. Typically, chains or branches of elongated connected cells are referred to as pseudohyphae [196], whereas cells containing defined septa between cells and no constrictions in their cell walls are known as hyphae [197]. Various different environmental cues induce the morphological transition from yeast to filaments. Many of these cues require a concurrent increase in temperature to 37 °C, and many of the cues mimic host physiological conditions. For example, serum is a potent inducer of filamentation at 37 °C, with key effectors being bacterial peptidoglycans and glucose [198–200]. At host physiological temperature, deprivation of nutrients such as carbon or nitrogen can induce a filamentous program, as can alkaline conditions (pH >6.5) or elevated CO_2 [201]. The requirement of 37 °C for filamentation to occur in many cues is due at least in part to the Hsp90-mediated repression of filamentation, which is relieved at elevated temperature due to global problems in protein folding that can overwhelm the functional capacity of Hsp90 [202]. Many cues induce filamentation in *C. albicans* via the cAMP–PKA signaling pathway, which activates transcription factors such as Efg1 [203] and Flo8 [204]. Filamentation is also governed by repressors such as Nrg1, whose downregulation and degradation is required for both hyphal initiation and maintenance [205].

The ability of *C. albicans* to transition between yeast and filamentous states is key for its dissemination and virulence in different niches within the host. Invasion of epithelial cells by *C. albicans* can occur by either endocytosis or by active penetration by hyphal cells [206]. Cells locked in the yeast form are reduced in virulence [207, 208], and defective in adhering to and invading oral epithelial cells [209]. Similarly, cells unable to revert to the yeast form also have attenuated virulence and show a decrease in kidney fungal burden in a murine model of systemic candidiasis [210]. It is thought that yeast cells are

required for dissemination throughout the body, while filamentous cells are essential for penetrating tissues, establishing infection and causing mortality [211]. A recent study has shown that a clinical isolate locked in the yeast form is more fit in a murine model of commensal infection [212]. Furthermore, another recent study demonstrated that cells locked in the yeast form are better able to colonize the gastrointestinal tract in a mouse model, while constitutively filamentous cells showed a decrease in colonization [213]. Taken together, this demonstrates that the polymorphic nature of C. *albicans* is a key virulence trait that allows it to adapt to the diverse conditions encountered within the host.

While both yeast and filamentous morphologies contribute to the pathogenesis of *C. albicans*, filamentous cells do possess specific virulence characteristics that increase their pathogenicity. For example, the sustained polarized growth exhibited by a filamentous cell provides a physical force that allows the fungal cell to penetrate host tissues, allowing for further invasion of tissues and organs [214]. Hyphal cells also express Als3, a hypha-specific protein found on the fungal cell surface [215]. Als3 is an adhesin that mediates binding to host cells, and induces endocytosis by binding to the host cell receptors E-cadherin and N-cadherin [215]. Hwp1 is another hyphal-specific fungal cell surface protein required to mediate attachment to epithelial cells [216]. In addition, the expression of genes encoding secreted aspartyl proteinases (SAPs) is coordinated with morphological state with *SAP4* and *SAP6* being expressed by hyphal cells [217, 218]. Additionally, connections exist between *C. albicans* filamentation and drug resistance. Certain proteins such as Hsp90 [219], O-mannosyltransferases [220], and the transcription factor Ndt80 [221] have roles in both morphogenesis and drug resistance.

In addition to the striking morphological differences between yeast and filamentous forms, *C. albicans* can also undergo distinct phenotypic switches. One prominent example is the switch between white and opaque cells. The more common white cells have a round or oval shape, while opaque cells are oblong and dimpled. This transition is governed by the regulator Wor1, with expression of *WOR1* leading to opaque cell formation [222]. Opaque cells are mating competent, with progeny showing recombination between chromosomes and alterations in ploidy [223, 224]. White and opaque cells show distinct preferences for different host environments and niches. Since opaque cells are not stable at 37 °C, they preferentially colonize surface environments such as the skin [225]. Furthermore, while white cells require the elevated temperature of 37 °C for filamentation, opaque cells filament preferentially at 25 °C [226]. Finally, white-opaque switching may aid in the evasion of host defenses, where white phase cells are preferentially recognized by neutrophils in certain environmental conditions [227, 228]. A novel phenotypic switch was recently discovered upon passage of wild-type white cells through the mouse gastrointestinal tract [117]. Induction of *WOR1* in this environment resulted in morphologically and functionally distinct GUT (gastrointestinally induced transition) cells, which are optimized for the digestive tract. A third phenotypic variant, the 'gray' phenotype has recently been identified [229]. This variant shows elevated levels of SAP expression and the propensity to colonize cutaneous tissue, similar to opaque cells. The master regulators Wor1 and Efg1 may act coordinately to regulate switching between white, gray and opaque cellular states [229].

Distinct morphological transitions are also important for virulence in *C. neoformans* (Fig. 1). While a typical *C. neoformans* cell is approximately 5–10 μM, specific conditions can induce the formation of titan cells, which can be as large as 100 μM [230]. Distinct features of titan cells include a thicker cell wall and denser capsule [231], as well as a tetraploid or octaploid DNA content [230]. Although the exact mechanism for titan cell formation is unclear, in part due to the difficulty in culturing titan cells in vitro [232], several signaling pathways have been implicated. The G protein-coupled receptors Ste3a (a pheromone receptor) and Gpr5 are required for titan cell production in vivo [170, 230] as is signaling through the cAMP/PKA pathway [170, 231]. The Rim101 transcription factor downstream of PKA signaling is also required for titan cell production in vivo [170]. Titan cells play a central role in *C. neoformans* pathogenicity, in that they interact with the host immune system. The large size of titan cells may prevent their phagocytosis [231, 233]; titan cells may also protect typical-sized cells from being phagocytosed [233], and promote dissemination from the lungs [234].

Biofilms

Biofilms are complex three-dimensional surface-associated communities of yeast and hyphal cells within an extracellular matrix (ECM), and can be found on medical devices such as catheters and artificial joints [235], or on mucosal surfaces [236], contributing to virulence (Fig. 1). The first step in biofilm formation is adherence to either an abiotic or biotic surface. In *C. albicans*, cell wall adhesins such as Eap1 and Als1, and other cell wall proteins have roles in attachment of the biofilm to the surface, which then stimulates changes in gene expression [237]. Adherence is followed by proliferation of yeast cells. This basal layer of yeast cells may contribute to anchoring the biofilm to the surface [238]. Next, yeast cells undergo the

morphogenetic transition to filamentous growth. The formation of hyphae is important, as strains defective in filamentation have a vastly reduced ability to form biofilms, as with mutants lacking a major transcriptional regulator of morphogenesis, Efg1 [238]. Accumulation of the ECM is part of the biofilm maturation process. The ECM is composed of mainly protein and carbohydrates, including mannans and glucans; however, lipids and nucleic acids are also found in the ECM [239]. Finally, yeast cells are dispersed from the biofilm. These cells have elevated pathogenicity, and an increased ability to adhere [237].

Biofilms are notoriously difficult to treat with antifungals, and have especially high levels of resistance to azoles and polyenes [237]. Resistance to azoles is dependent on the molecular chaperone Hsp90 [45], glucan modification enzymes that are required for delivery and organization of the ECM [240], and β-1,3 glucan in the ECM that may sequester azoles [241]. Increased expression of efflux pumps early in biofilm development, and the presence of persister cells also contribute to antifungal resistance [242].

Secreted factors

Secretion of proteinases such as SAPs is crucial for both pathogenesis and nutrient acquisition. As mentioned above, *C. albicans SAP4* and *SAP6* expression is coordinated with hyphal formation [217, 218], while expression of *SAP1* is regulated by the white-to-opaque phenotypic switch [243]. During infection, the SAP proteins have roles in adherence to host cells [244] and degradation of host proteins such as mucin [245], perhaps allowing penetration and colonization of tissues. SAP proteins may also degrade host antimicrobial factors such as lactoferrin, complement and the proteinase inhibitor cystatin A [244]. Secreted phospholipases cleave ester linkages in glycophospholipids, and can have roles in host cell penetration and invasion [246]. Mutants lacking the phospholipase Plb1 have reduced virulence in a murine model of *Candida* infection [247].

Histoplasma also secretes several factors important for pathogenesis. The calcium-binding protein Cbp1 is secreted from yeast-phase *H. capsulatum*, and is required for growth in calcium-depleted conditions and for virulence in a murine model of pulmonary histoplasmosis [248]. Resolution of the structure by NRM revealed that Cbp1 may be a lipid-binding protein, and possibly interacts with glycolipids in the host [249]. Yps3 is both a secreted factor as well as a cell wall protein in *H. capsulatum*, and while its exact function is yet to be elucidated, silencing of *YPS3* results in reduced organ colonization in a murine model of infection [250].

Evasion of the host immune system

Capsule and cell wall

The capsule of *C. neoformans* is an important virulence determinant, playing key roles in surviving environmental conditions and modulating the host immune response (Fig. 1). The capsule is primarily composed of the polysaccharides glucuronoxylomannan (GXM) and glucuronoxylomannogalactan (GXMGal) [251]. Monomers of simple sugars are modified and polymerize within the cell before being secreted and attached to the cell wall surface. An important modification of capsule monomers is xylosylation, such that deletion of the xylosyltransferase gene *CXT1* results in an altered capsule structure and reduced virulence [252]. O-acetylation contributes to the antigenicity of the capsule by affecting binding of antibodies and activation of the complement cascade [253]. Hyaluronic acid, synthesized by Cps1, is also added to the capsule and may play a role in facilitating passage of *C. neoformans* across the blood–brain barrier [254]. Finally, antigenic variation of capsule monomers can lead to differential binding of antibodies [255].

Just as conditions that mimic physiologically relevant host conditions induce filamentation in *C. albicans*, similar cues also induce capsule formation in *C. neoformans*. Upon infection of a host, the capsule increases in size with the thickness being determined by the location of infection. Infection of the lungs results in a thicker capsule than does infection of the brain [256], demonstrating that regulation of capsule synthesis is dependent on the environmental niche. Nutrient-poor conditions such as low glucose and low nitrogen can contribute to capsule induction by signaling through the cAMP–PKA pathway [257–259]. Similarly, as iron levels are tightly controlled within the host, conditions of low iron are a potent inducer of capsule formation [260]. The iron-sensing transcription factor Cir1 is one of the main regulators of capsule induction in response to iron poor conditions [183]. Induction of capsule formation in low iron conditions is also signaled through the cAMP–PKA pathway [251]. Additionally, the cAMP pathway is used to signal capsule formation in the presence of high CO_2 levels [251, 261]. Physiological pH is another cue that is sensed by *C. neoformans* to regulate capsule formation, and the conserved Rim101 pH-sensing pathway integrates signals from the cAMP–PKA pathway to regulate attachment of the capsule to the fungal cell wall [169]. However, it appears that alkaline pH alone is insufficient to induce an enlarged capsule, and instead this condition must be combined with others, such as nutrient deprivation or the presence

of serum [262]. Finally, stress response pathways can have a repressive effect on capsule induction, such as the Hog1 and protein kinase C (PKC) pathways that respond to osmotic stress [251].

The capsule is essential for *C. neoformans* interactions with the host immune system. The polysaccharides GXM and GXMGal have immunosuppressive properties such as modulation of macrophage, neutrophil and dendritic cell activities, and also inhibition of pro-inflammatory cytokine production [263]. GXMGal reduces B cell activity and inhibits activation of T cells. The capsule also has antiphagocytic properties, inhibiting phagocytosis in vitro due to the large size of the capsule, and possibly by hiding pathogen-associated molecular patterns (PAMPs) on the cell surface [264].

To avoid interaction with host phagocytes that would initiate the production of reactive oxygen species and the secretion of pro-inflammatory cytokines, *H. capsulatum* antigenic cell surface β glucans are hidden under a layer of α-(1,3)-glucan. Silencing of *AGS1,* encoding the α-glucan synthase, severely attenuates virulence of *H. capsulatum* in mouse lungs [265]. Loss of α-(1,3)-glucan allows for recognition of the β glucans by the host receptor dectin-1 [266]. Additionally, heat shock protein 60 (HSP60) on the cell surface binds to the CR3 receptor on macrophages, followed by phagocytosis [267]. However, interactions with CR3 do not typically result in a strong immune response unless other costimulatory signals are present [268, 269], allowing *H. capsulatum* to grow and survive within macrophages.

Escape from macrophages

Fungi have evolved multiple mechanisms to avoid being killed upon phagocytosis by immune cells such as macrophages. One mechanism is to escape the phagocyte, either by inducing macrophage lysis or by non-lytic exocytosis. Non-lytic expulsion of fungal cells was first described in *C. neoformans*, where live yeasts were able to escape macrophages without killing the host cell [270–272]. This prevents release of pro-inflammatory cytokines and potentially aids in transversal of cryptococcal cells across the blood–brain barrier [105]. This phenomenon of non-lytic expulsion has also been observed in *C. albicans,* although at lower frequency than for *C. neoformans* [273]. *Candida albicans* can also escape from macrophages via filamentation. These filaments can induce pyroptosis, a programmed pro-inflammatory macrophage death [274, 275]. This process is hypothesized to be dependent on the transition to hyphae, which stimulate host pyroptotic caspases and result in macrophage death [274, 275].

Nitric oxide detoxification

Nitric oxide (NO) is a nitrogen radical and antimicrobial effector produced by the host immune system in response to fungal infections. *Candida albicans* responds to this stress by increasing the expression of an NO dioxygenase flavohemoglobin, *YHB1* [276, 277], which metabolizes NO. *Candida albicans* cells lacking *YHB1* have increased sensitivity to NO and reduced virulence in a murine model of systemic candidiasis [276, 277].

Cryptococcus neoformans also employs an enzymatic defense against NO produced by the host. The flavohemoglobin denitrosylase Fhb1 consumes NO, and cells in which *FHB1* is disrupted have reduced survival in macrophages and decreased virulence in a murine inhalation model [278]. A global analysis of the *C. neoformans* response to nitrosative stress revealed that the glutathione reductase Glr1 is upregulated in response to NO [279]. Deletion of *GLR1* renders *C. neoformans* sensitive to nitrosative stress, and avirulent in a mouse inhalation model [279].

Although the presence of a flavohemoglobin in *H. capsulatum* is not apparent, a shotgun genomic microarray did identify *NOR1*, a P450 nitric oxide reductase homologue, whose expression is increased by nitrosative stress [280]. Overexpression of this gene decreased the sensitivity of *H. capsulatum* to reactive nitrogen species [280].

Melanin

In *C. neoformans*, melanins are pigmented molecules with antioxidant properties, synthesized by the laccase enzymes Lac1 and Lac2 [264]. Melanization occurs during infection [281], and overexpression of melanin results in decreased recognition by the host and modulation of host cell immune responses [282]. Melanized cells are also less susceptible to microbicidal peptides and recognition by macrophages, as well as being less susceptible to antifugal drugs in vitro [283]. Together, this demonstrates an important role for melanin in the virulence of *C. neoformans*.

Urea metabolism

In addition to being used as a nitrogen source by many fungi, *C. albicans* and *C. neoformans* also use urea metabolism to modulate the host immune system and alter dissemination [284]. Urease is considered a cryptococcal virulence factor as urease expression is linked with increased invasion across the blood–brain barrier [285], increased fungal burden, and altered host immune responses [286–288]. Urease-positive *C. neoformans* induce a strong non-protective Th2 response as demonstrated by

an increased accumulation of eosinophils in the lung, increased serum IgE and higher levels of Th2 cytokines [288], demonstrating a role for urease in influencing the host immune response.

While *C. albicans* does not contain a urease enzyme, it does contain a urea amidolyase, encoded by *DUR1,2* [289]. Deletion of *DUR1,2* results in cells that are less virulent than wild-type cells in a murine model of disseminated candidiasis and results in decreased kidney fungal burden [290]. Additionally, deletion of *DUR1,2* causes a decreased inflammatory response in the kidneys, reduced neutrophil infiltration and altered host cytokine and chemokine production, suggesting a role for urea metabolism in modulating host immune responses [290]. A *dur1,2* mutant also shows impaired escape from host macrophages as it is unable to form filaments in the presence of urea or arginine, a urea-precursor [291]. This may be coupled to the defect of the *dur1,2* strain in auto-alkalinization of the environment [174].

Host immune responses

The interactions between opportunistic fungal infections and the host immune system are complex and only beginning to be understood. Outcomes of infections are dependent on interactions between the host immune response and the intrinsic virulence of the pathogen. Opportunistic fungi such as *C. albicans* constantly interact with the host immune system, and perturbations to this balance can result in a switch from commensalism to pathogenesis, as discussed above. Alterations to the innate or adaptive host immune responses, either due to immunosuppression or genetic predisposition, can result in severe and often deadly fungal infections. The interplay between fungi and the host immune system has been the subject of several recent reviews [8, 292–294]. Here we summarize this interaction, and describe host factors that can increase susceptibility to fungal infections.

Host response to fungal pathogens

Innate

Physical barriers such as the skin and mucosal epithelial cells at sites continuously in contact with fungal pathogens are the first line of defense against fungal pathogens. The next barrier is the innate immune response, which is crucial for preventing invasive and systemic infections. The innate immune response is required for recognition of fungal PAMPs. The fungal cell wall is composed of many PAMPs, including both α- and β-glucans, chitin, chitosan, and mannans [295]. These PAMPs can be recognized by host pattern recognition receptors (PRRs) on phagocytic cells such as macrophages, dendritic cells and neutrophils [292, 293]. Specific PAMPs on different fungal species can be recognized by specific PRRs, including complement components, toll-like receptors (TLRs), C-type lectin receptors (CLRs), and mannose receptors (MR), among others [296–299]. These interactions will shape the resulting immune response, including phagocytosis of the pathogen, production of inflammatory cytokines, and activation of T cells.

Upon phagocytosis, fungal cells can be killed by effector molecules such as proteases, defensins, and cationic peptides as well as by the production of reactive oxygen and nitrogen species [294]. Neutrophils are highly effective at containing fungal infections, including inhibition of the *C. albicans* morphological transition [300]. Neutropenic patients, including those with acute leukemia, are at high risk of developing invasive *Candida* and *Aspergillus* infections, and are often given prophylactic antifungal therapies as a preventative measure [301]. However, increased neutrophils in mouse kidneys are associated with pathogenesis of *C. albicans* at late time points [302].

Adaptive

The adaptive immune system involves stimulation of T cells by antigen-presenting cells [292, 303]. These antigen-presenting cells can generate specific cytokine profiles that can bias the immune system towards Th1-, Th2-, Treg-, or Th17-responses [303]. The balance between these responses is crucial for the ability to clear the pathogen without causing autoimmune damage.

The adaptive immune response plays a pivotal role in preventing fungal infections, as highlighted by the incidence of fungal disease in AIDS patients [10, 304]. HIV infection depletes the pool of $CD4^+$ T cells, consequently causing a loss of antifungal immunity. This results in impaired production of interferon-γ (IFN-γ) and tumor necrosis factor-α (TNF-α) [304]. Additionally, the population of memory B cells is depleted [305], and macrophage and dendritic cell function is impaired upon HIV infection [306], contributing to increased susceptibility to fungal infections.

Th1 responses include the production of IFN-γ, which is key for controlling many disseminated fungal infections [303]. *Cryptococcus neoformans* strains that induce Th1-biased responses are generally cleared from the lung and do not cause disease [307, 308]. The decrease in Th1-type cell-mediated immune responses in AIDS patients may explain their increased susceptibility to cryptococcal infections compared to patients with other types of immune deficiencies [309].

Th17 responses appear to be critical for host defense against fungal infections, especially *Candida* [310]. In HIV patients, susceptibility to mucosal candidiasis can be attributed to a loss of mucosal Th17 cells, which is accompanied by a decrease in the integrity of epithelial cells and alterations to the intestinal environment [311]. Decreases in the Th17 response also leads to increased severity of *Pneumocystis* infections in mice [312].

Regulatory T (Treg) cells continually survey the host for signs of mucosal infections and aid in preventing reinfection. However, Treg cells are able to suppress inflammation during systemic candidiasis by producing interleukin 4 (IL-4), IL-10, and transforming growth factor-β (TGF-β), which inhibit inflammatory Th1 and Th17 responses making them detrimental for clearing the infection [313]. Depletion of Treg cells results in decreased *C. albicans* growth in a murine model of candidiasis [313], and a decrease in Treg cells is also associated with an increase in the Th17 responses that are beneficial in clearing *Histoplasma* infections [314].

In contrast, Th2 responses to fungal infections are often deleterious [292]. During *C. neoformans* infections, the fungi are able to induce a strong Th2-biased immune response, with increased levels of IL-4 and IL-5, thus favoring persistence of the infection [309]. Mice with increased IL-4 levels also demonstrated increased *Histoplasma* infections [315]. In addition to favoring persistence of the infection, Th2 responses are also linked with allergic bronchopulmonary aspergillosis and increased susceptibility to invasive aspergillosis [316]. In contrast, Th2 responses may be protective against *Pneumocystis* pneumonia, with affected HIV individuals showing low levels of Th2 cytokines [317].

Immune reconstitution inflammatory syndrome

The recovered immune system in AIDS patients treated with antiretrovirals can overreact to PAMPs exposed during fungal infections, even if the infections have been cleared by antifungal therapies. This overactive inflammation is known as immune reconstitution inflammatory syndrome (IRIS), and it can cause significant mortality due to damage by the host immune system. IRIS is a major consideration in the treatment of AIDS patients with cryptococcosis. Recent studies have noted that between 8 and 50 % of AIDS patients develop cryptococcal IRIS, even when no live cryptococcal cells are recovered from the patient [35, 318]. IRIS can also occur in transplant patients after reduction in the immunosuppresive therapies used to prevent organ rejection [319]. The inflammatory immune response in these IRIS patients consists of increased IL-6 and C-reactive protein (CRP) levels. Additionally, many patients who go on to develop IRIS have decreased TNF-α and other Th1 cytokines prior to antiretroviral therapy [320]. The current standard of care for IRIS patients includes administration of steroids to decrease systemic inflammatory responses and prevent further damage to host tissues [36, 90].

Genetic susceptibility to fungal infections

Chronic mucocutaneous candidiasis

Recurrent *Candida* infections of cutaneous or mucosal surfaces in the absence of immunosuppressive conditions (such as HIV), is described as chronic mucocutaneous candidiasis (CMC). IL-17 signaling and activation of T cells are essential for defending against mucocutaneous *C. albicans* infections and genetic mutations that perturb this signaling pathway can lead to CMC [321].

Numerous single nucleotide polymorphisms have been associated with increased susceptibility to CMC (see [8, 292, 321]). For example, loss of function of the CLR dectin-1 can lead to increased onychomycosis and mucocutaneous candidiasis [322, 323]. This is the result of defective recognition of β glucan in the *C. albicans* cell wall and impaired cytokine production (including IL-1β [323] and IL-17 [322]). Neutrophil function remains unaffected, and thus systemic candidiasis is not associated with dectin-1 mutations [323]. The caspase recruitment domain-containing protein 9 (CARD9) is a signaling protein downstream of many CLRs, including dectin-1 [321] and activates the nuclear factor-κB (NF-κB) pathway [324]. Autosomal recessive mutations in CARD9 can result in decreased development of Th17 cells and is associated with CMC [324]. Autosomal dominant gain-of-function mutations such as those in STAT1 (signal transducer and activator of transcription 1) can also lead to inhibition of Th17 development, and increased susceptibility to CMC [325, 326]. Additionally, autosomal recessive mutations in the IL-17 receptor IL-17RA, and deficiencies in the cytokine IL-17F can result in CMC [327]. Mice with deficient IL-17A production show an impaired ability to clear a cutaneous *C. albicans* infection [328], suggesting that both IL-17A and IL-17F are required for the defense against chronic mucocutaneous candidiasis.

Additional syndromes can lead to CMC. One of these is hyper-IgE syndrome (HIES), in which patients experience recurrent pulmonary infections, eczema, staphylococcal infections, and CMC, among other symptoms. Mutations in *STAT3* have been identified as a cause of HIES [329], in which an inability of $CD4^+$ T lymphocytes to differentiate into Th17 cells results in impaired IL-17 production [330]. Mutations in DOCK8 (dedicator of cytokinesis 8) have been identified in the autosomal recessive form of this syndrome, in which there is defective T cell activation and Th17 cell

differentiation [331]. Finally, the rare autoimmune polyendocrinopathy–candidiasis–ectodermal dystrophy (APECED) syndrome is characterized by development of CMC in almost all patients, and is caused by mutations in the autoimmune regulator AIRE [321, 332]. The strong prevalence of CMC in these patients may be due to the production of neutralizing autoantibodies against Th17-produced cytokines, specifically IL-17A, IL-17F and IL-22 [333].

Invasive fungal infections

While mucocutaneous *Candida* infections are primarily caused by defects in the adaptive immune system, deficiencies in the innate immune response, such as those caused by primary immunodefiencies can lead to invasive yeast infections. A few such cases are outlined below. Chronic granulomatous disease (CGD) is the result of mutations in genes encoding components of the NADPH oxidase complex, impairing the ability of phagocytes to produce reactive oxygen species [334]. While the primary fungal infection associated with CGD is invasive aspergillosis, [335] infections by *Candida* and *Trichosporon* species have also been noted [321]. Myeloperoxidase (MPO) deficiency is a disorder in which the production of antimicrobial hypochlorous acid by phagocytic cells is impaired, and mice deficient for either NADPH or MPO show increased mortality in response to a high dose of *C. albicans* [336]. While the majority of MPO patients do not suffer from fungal infections, those who do acquire infections usually also have diabetes [337]. Patients presenting with monocytopenia are more susceptible to histoplasmosis and cryptococcal infections [338], and cryptococcosis has also been observed in patients with hyper-IgE or hyper-IgM syndromes and idiopathic CD4 lymphocytopenia [321]. Finally, patients with severe combined immunodeficiency commonly acquire *Pneumocystis* pneumonia, as do those with hyper-IgM syndrome [321].

Management of fungal infections

Diagnosis

The first step in achieving the proper management of infectious disease is the effective diagnosis and species identification of the pathogen. Unfortunately, shortcomings in current diagnostic techniques remain one of the greatest challenges in the field. In most cases, diagnosis still relies on traditional culture methods and histopathology [10, 87, 339, 340]. Culturing the organism and subsequent identification is a slow process that takes several days, leading to considerable delays in treatment. Such delays have direct and significant impact on disease outcome and patient mortality, especially for severe invasive diseases [136, 340]. A number of novel molecular diagnostic approaches have been developed to improve the current system, including PCR-based assays and antigen detection systems [339, 341]. However, these are not regular practice in the clinic, and still need to be standardized and tested in large patient cohorts before they can be incorporated into clinical guidelines [87, 90, 110, 339].

Antifungal drugs

Due to the close evolutionary relatedness between humans and fungi, the number of targets that can be selectively exploited for drug development is limited [10, 342]. There are also very few drugs currently being developed, primarily because antifungals are not predicted to generate a large enough financial return for pharmaceutical companies [10]. Polyenes, azoles, and echinocandins represent the three most common classes of antifungals currently used in the clinic, each with their own advantages and limitations. Overall, host toxicity, cross reactivity with other drugs, and development of drug resistance pose great challenges to current antifungals.

Polyenes

Polyenes are broad-spectrum natural product antifungals discovered in the early 1950s from the bacterial genus *Streptomyces* [342, 343]. Polyenes bind to ergosterol in the fungal membrane, generating aqueous pores that result in leakage of fungal cell content and eventually cell death [344, 345]. Amphotericin B, one of the most successful polyene derivatives, was a gold standard in treating serious fungal infections. However, amphotericin B is known to cause severe systemic toxicity and nephrotoxicity [342, 346]. Various drug delivery systems have been developed to improve its safety profile, including the popular lipid formulation [347, 348]. While its clinical use decreased with the development of azoles in the late 1980s, it is still widely deployed to treat life-threatening disseminated and invasive mycoses [90, 110]. Fortunately, despite its long history of clinical use, resistance to polyenes remains a rare occurrence [349, 350].

Azoles

Azoles are synthetic compounds that were first introduced as antifungals in the late 1980s and early 1990s [344]. Their low toxicity led to extensive use in the clinic. Azoles disrupt the biosynthesis of ergosterol by inhibiting the cytochrome P-450-dependent enzyme lanosterol

demethylase (also referred to as 14α-sterol demethylase) [351]. Azoles are chemically classified as either imidazoles if they have two nitrogen atoms in the azole ring, or triazoles if they have three [343, 351]. Imidazoles are typically limited to topical treatment of superficial infections, while triazoles are used more broadly in both superficial and systemic infections due to their superior pharmacokinetic and safety profile. The most commonly used azoles in the clinic include fluconazole, itraconazole, voriconazole, and posaconazole.

Azoles remain the drug of choice as initial therapy for most fungal infections and are often recommended as prophylaxis for high-risk patients [90, 110, 352]. Widespread use of azoles has led to increasing reports of azole resistance in the clinic, which is associated with greater treatment difficulties and patient mortality [353]. There has also been an increased incidence of infections caused by intrinsically azole-resistant fungal species, including *C. glabrata* and *C. krusei*, creating major challenges for future treatments [87].

Echinocandins

Echinocandins first entered the market in 2001, representing the newest class of antifungals to reach the clinic [354]. Echinocandins are large semi-synthetic lipopeptides that inhibit the cell wall enzyme complex β-1,3-D-glucan synthase [355], thereby decreasing the concentration of β-(1,3)-glucan in the fungal cell wall and causing the subsequent loss of cell wall integrity. Echinocandins are generally well tolerated with little to no side effects [356].

Echinocandins are active against *Candida* and *Aspergillus* species, including azole-resistant *Candida* isolates, but show no in vivo activity against *Cryptococcus* [90, 110, 342]. Currently available echinocandins include caspofungin, anidulafungin, and micafungin [354]. All three are not orally bioavailable and need to be administered intravenously. Echinocandin resistance has been reported in the clinic, and the incidence appears to be on the rise [357].

Vaccines

An ideal solution to fungal management is the prevention of disease through vaccination. However, there is currently no clinically available vaccine for any fungal pathogen. Over the years, a number of vaccines have been in development against different fungi, with promising results in animal models [10, 358]. However, only a few have been translated to human clinical trials, with the major barrier being the lack of funding [359]. Efficacy trials are also difficult to conduct as high-risk patients often routinely receive antifungal prophylaxis [10]. Nonetheless, two subunit vaccines against *Candida* have recently demonstrated success in Phase I clinical trials, one of which is currently being tested in a Phase II trial in the United States [360]. This vaccine represents a promising advance and may stimulate further development of much needed immunotherapeutics and vaccines [358, 361, 362].

Concluding remarks

A significant advance in our understanding of invasive fungal infections has been made in recent years, motivated at least in part by the increasing incidence of these infections. Given the growing number of transplant patients, those in receipt of immunosuppressive therapy and the global HIV pandemic, this comes as no surprise. Host niches are complex, dynamic and distinct to each individual, as are the pathogens' response strategies for coexisting with or evading the host immune response. Taking steps towards understanding the underlying immunopathogenesis of fungal infections permits early and accurate diagnosis and treatment. We must continue to seek out novel diagnostics that will allow for rapid treatment of fungal infections with the appropriate antifungal. With the rise in antifungal drug resistance now a real threat, researchers must strive to discover novel antifungals and antifungal drug combinations, as well as effective immunotherapeutic strategies that combat resistance, improve patient outcome, shorten hospital stays and ease the economic burden.

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