The pathobiology of human fungal infections

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Abstract

Human fungal infections are a historically neglected area of disease research, yet they cause over 1.5 million deaths every year. Our understanding of the pathophysiology of these infections has increased considerably over the past decade, through major insights into both the host and pathogen factors that contribute to the phenotype and severity of these diseases. Recent studies are revealing multiple mechanisms by which fungi modify and manipulate the host, escape immune surveillance and generate complex co-morbidities. Although the emergence of fungal strains that are less susceptible to antifungal drugs or that rapidly evolve drug resistance are posing new threats, greater understanding of immune mechanisms and host susceptibility factors are beginning to offer novel immunotherapeutic options for the future. In this Review, we provide a broad and comprehensive overview of the pathobiology of human fungal infections, focussing specifically on pathogens that can cause invasive life threatening infections, highlighting recent discoveries from the pathogen, host and clinical perspectives. We conclude by discussing key future challenges including antifungal drug resistance, the emergence of new pathogens and new developments in modern medicine that are promoting susceptibility to infection.

[H1] Introduction

Of all classes of microorganisms that can infect humans, fungi are amongst the least studied and understood. Yet their cumulative impact on human health is staggering, as fungal infections affect billions of people and result in more than 2 million deaths every year [1]. The publication of the fungal pathogen priority list by the World Health Organisation in 2022 [2] was a major acknowledgement of the importance of these pathogens to human health (Figure 1, supplementary Table S1) and the difficulties we face in tackling the diseases they cause, including the limited diagnostic and therapeutic options currently available (Table 1). Over the past decade there have been considerable increases in our knowledge and understanding of the pathogenesis of fungal infections. New discoveries have revealed startling and unexpected insights into the underlying mechanisms of fungal virulence and host immunity, and their impact on human health. In this Review, we integrate these exciting recent advances into a comprehensive overview of fungal pathobiology from the pathogen, host and clinical perspectives. Although superficial fungal infections of the nails, hair and skin, and allergic fungal diseases can have a huge impact on human morbidity, the primary focus of this Review is on fungi that can cause invasive life threatening infections.

[H1] Fungal virulence

[H2] The evolution of virulence.

Only a small proportion of fungal species (about 600) have been reported to cause infections in humans [3]. The ability of fungi to infect humans has emerged numerous times, independently across the kingdom [4], spanning millions of years of evolution. Indeed, nearly half of fungal phyla include species able to infect humans. Increasing numbers of fungi are emerging as aetiological agents of disease in humans as a result of increased human exposure to the pathogens, larger populations with host immune impairments, as well as fungi exhibiting increased pathogenicity and/or by colonising new ecological niches (geographically or zoonotically) [4] (BOX 1). For example, the recent emergence of *Candida auris*, is causing concern in healthcare facilities worldwide [5].

Despite their evolutionary diversity, fungal pathogens of humans display common phenotypic properties that reflect the biotic and abiotic challenges imposed by mammalian host tissues. These common phenotypes include thermotolerance, which is an important prerequisite to be able to cause infections in humans. Whether they are principally saprobic (such as *Aspergillus, Histoplasma* or *Talaromyces* species), form part of the commensal microbiota (such as *Candida* species and other related yeast genera) or are obligately associated with their human host (for

example, *Pneumocystis jirovecii*), fungi that are capable of causing human infections must be able to tolerate the ambient temperature of the site or sites they infect [4]. This is of importance, as the global climate crisis may select for adaptive thermotolerance, which might lead to the emergence of new fungal pathogens, as well as increasing the geographical spread of extant fungal pathogens.

[H2] The role of fungal morphology in virulence. Fungi display a wide variety of morphological forms, and their ability to switch forms can have a key role in virulence (Figure 2). For example, *C. albicans* is polymorphic and forms yeast, pseudohyphae, hyphae and chlamydospores, as well as the yeast-like morphologies opaque and gray [6]. This organism is a common human commensal and is found in more than 80% of individuals [7] but can also cause mucosal as well as life-threating invasive infections in certain situations. The yeast–hypha transition has received widespread attention, with earlier work demonstrating its importance in disseminated disease, as well as roles in tissue invasion and destruction, and biofilm formation. *C. albicans* hyphae have direct roles in virulence through induced endocytosis [8], thigmotropism [9] and active penetration driving invasion [9,10], the formation of trans-cellular tunnels [11], and escape from macrophages [12]. In addition, genes co-regulated with hyphal development have a key role in virulence, encoding factors such as adhesins (for example, Als3 (Ref. [13]) and Hwp1 (Ref. [14])), hydrolytic enzymes (for example, Sap4-6 (Refs.[15]), Sod5 (Refs. [16,17])) and the hypha-specific cytolytic peptide toxin, candidalysin (derived from Ece1), which might be involved in nutrient acquisition from the host [18]. Nonetheless, the close association between the yeast–hypha transition and virulence has been brought into question, with recent work demonstrating that metabolic flexibility and cell wall remodelling can overcome the impact of filamentation defects [19,20]. However, the ability to undergo transitions between yeast and hyphal forms is key to its success as a commensal and pathogen [21-23]. The yeast-like morphologies, opaque and gray, may also have a more specific role in virulence in certain microenvironments, as these cells seem to be better adapted to colonise the skin, tongue and gastrointestinal tract [24].

In contrast to *C. albicans*, thermally dimorphic fungi, such as *Paracoccidioides* spp., *Talaromyces marneffei, Histoplasma* spp. and *Coccidioides* spp., switch from their environmental hyphal form to the pathogenic yeast form or, for *Coccidioides* spp., a spherule tissue form, in the host, mainly driven by temperature. These thermo-dimorphic switches are generally co-regulated with cell wall remodelling and the expression of secreted factors that have a role in virulence; such as *Blastomyces* virulence adhesin-1 (BAD1 Ref [25]) and *Coccidioides* spherule outer wall fraction glycoprotein (SOWgp) [26], which promote adhesion, and *Histoplasma capsulatum* calcium-binding protein 1 (CBP1) that promotes phagocyte cell death [27]. In the host, *Cryptococcus* species become encapsulated yeasts and have the capacity to form large polyploid cells,

termed ‘titan’ cells [28-30]. The balance between yeast and titan cells in the lung is key to virulence [31], and is discussed in more detail below. Filamentous fungal pathogens such as *Aspergillus*, *Fusarium* and *Mucorales* spp. also undergo morphological changes linked to cell wall remodelling and protein secretion during host colonisation. However, unlike the aforementioned examples of inducible morphogenesis, the spore-to-hypha germination in filamentous fungi is an obligatory feature of proliferation and tissue invasion.

[H2] The role of secretory products in virulence. The secretion of molecules into the extracellular environment is a fundamental property of the fungal kingdom required for the enzymatic degradation of large molecules for nutrient acquisition. For human fungal pathogens, many secreted factors can drive the infection process and contribute to immune evasion (Figure 2). Fungal genomes often carry genesthat encode secreted factors in higher copy numbers, and greater diversity compared to their non-pathogenic relatives. One of the best characterised examples is the family of secreted aspartic proteases (Saps) of *C. albicans*, several of which are released into the extracellular environment [15]. As well as processing proteins to function as a nutrient source (predominantly nitrogen), many Saps also modulate host immunity, for example by degrading antimicrobial peptides and components of the complement cascade. Similarly, *A. fumigatus* Mep1 and *C. neoformans* App1 cleave complement components [32]. *C. neoformans* secretes an M36 metalloprotease, Mpr1, which mediates fungal traversal of the blood–brain barrier [33]. Although such proteases are not unique to pathogens, these species often have larger protease gene families, which indicates positive selection in human (and other animal) pathogens [34]. *C. albicans* also secretes a lipase, Lip2, that alters host tissue lipids, which results in suppression of Th17 responses[35]. In addition to secreted proteins, many fungi produce and secrete small-molecule secondary metabolites, which have a wide range of activities: penicillin is a well-known example of a secondary metabolite antibiotic. Others function in micronutrient scavenging (siderophores, discussed below) and immune modulation [36] . *Aspergillus* and *Fusarium* species also produce small-molecule toxins including gliotoxin, aflatoxin and fumonisins [37].

To proliferate in the infected host, fungi must secure micronutrients such as iron and zinc. Indeed, infections with *Mucorales* spp. are strongly associated with iron overload [38,39]. Fungal acquisition of these micronutrients is particularly challenging because the immune competent host actively restricts access to these trace minerals via nutritional immunity. To capture iron from the host, *A. fumigatus* relies upon intracellular (ferricrocin and hydroxyferricrocin) and extracellular (fusarinine C and triacetylfusarinine C) siderophores [40], whereas *C. albicans* cannot produce

its own siderophores, instead utilising host iron-binding protein scavengers including a ferritin receptor and a multi-member family of haem-binding proteins [41,42].[38]. Most other pathogenic fungi, including *Histoplasma capsulatum,* also compete with the host for zinc [43,44].

Beyond classic secreted proteins, many human fungal pathogens release extracellular vesicles (EVs). First identified in *C. neoformans*, EVs are proposed to act as 'virulence-bags', by trafficking proteins and other biomolecules [45]. EVs are likely to play a key role in fungal pathogenicity in humans. Indeed, *C. albicans* EVs were recently shown to induce type I interferon signalling through the cGAS–STING pathway [46]. However, the exact role of EVs in fungal cell surface remodelling, nutrient acquisition and export of virulence factors is still under investigation. As well as being released by fungi into their environments, EVs can traverse the cell wall in the opposite direction and be taken up by fungal cells. In this context, EVs may play key roles in inter-cellular communication [47,48]. *C. albicans* EVs have recently been shown to contribute towards the extracellular matrix production of biofilms. Indeed, endosomal sorting complexes required for transport (ESCRT)– mutants, that are defective in EV production, display highly reduced resistance to antifungals (a clinically important property of biofilms). Intriguingly, the addition of exogenous EVs to these defective biofilms reverses this effect, strongly suggesting that EVs directly contribute towards biofilm-mediated drug resistance [49].

[H2] Fungal fitness. Fungal pathogenicity is also dependent on 'fitness attributes' that enhance adaptation to, and thereby physiological robustness within, host niches. In addition to micronutrient scavenging and thermotolerance, described above, fitness attributes include metabolic adaptation; adaptation to local pH, oxygen and carbon dioxide levels; and the activation of robust responses to local environmental stresses such as hypoxia [50] and reactive chemical species delivered by local innate immune defences. For example, pathogens that evolved in environmental niches have developed metabolic flexibility and environmental stress resistance to pivot between periods of starvation and active recycling of environmental detritus, when available. Indeed, fatty acid metabolism and thermotolerance have become tightly linked in *A. fumigatus* through functionality of the heat shock transcription factor [51]. Similarly, pH-dependent expression of germination- and virulence-specific genes are implicated in a metabolic shift that enables survival of *Mucor circinelloides* within macrophages [39]. Meanwhile, fungal pathogens that have co-adapted with their host seem to have tuned their metabolic flexibility accordingly. For example, in contrast to biotechnological strains of *Saccharomyces cerevisiae,* which assimilate simple sugars before switching to less efficient carbon sources, clinical isolates of *S. cerevisiae* and *C. albicans* have the capacity to assimilate sugars and alternate carbon sources simultaneously, thereby enhancing their pathogenicity [52].

Conversely, *P. jirovecii* has become so dependent on its human host that it has lost biosynthetic pathways and corresponding regulatory transcription factors required for the synthesis of most amino acids as well as certain lipids, cofactors and vitamins [53].

Fungal pathogens also display a wide range of stress sensitivities. Host defences include the generation of reactive oxygen species (ROS), which promote apoptosis-like programmed cell death (A-PCD) in *C. albicans* and *A. fumigatus* [54,55]. The clinical significance of ROS-mediated fungal killing is exemplified by the sensitivity of individuals with reduced ROS-generating capacity (for example, with defective NADPH oxidase 2) to fungal infections [56]. Not surprisingly, therefore most fungal pathogens are resistant to host-derived ROS. *Nakaseomyces glabratus* (formerly *C. glabrata*), in particular, is extremely resistant to oxidative stresses even relative to other fungal pathogens, which is thought to be due to the evolutionary assimilation of novel stress-protective proteins [57]. At the other extreme, *P. jirovecii* has lost key transcription factors that, in other fungi, promote resistance to environmental stresses [53], and this is likely to contribute to the difficulties in culturing the organism *in vitro*.

[H2] Genetic variation. Fungal pathogens can infect diverse hosts and ecological niches, which is facilitated by the rapid responses of their genomes to natural selection [58]. Many examples of this have been described, including horizontal transfer of whole chromosomes (for example, *Fusarium oxysporum*, although the impact of horizontal transfer has only been shown in plants) [59], repeat-driven genome expansions [60] and structural variation [61], all of which are associated with host adaptation and changes in virulence across lineages and species. Maintaining sufficient genetic diversity to respond to selection is facilitated by the ability of fungi to use multiple reproductive modes, including cryptic recombination that enables inbreeding, outcrossing, hybridization and the generation of diversity via parasexual mechanisms [62].

Mechanisms increasingly recognised as driving dynamic genome structure in pathogenic fungi of humans include chromosomal copy-number variation (CCNV; also known as aneuploidy) [63], and the duplication of chromosome arms (isochromosomes). The mechanism or mechanisms that generate CCNV in fungi remains unclear, but exposure to environmental and host stresses enhances genome instability, and thereby evolutionary adaptability [64]. A change in ploidy or chromosome number can change how a cell senses and responds to its environment. Ploidy changes in fungi not only result in changes in cell size, growth rates, gene expression, adaptation to host niches and antifungal resistance but can also have longer term effects on genetic changes and potentially the evolution of novel traits. Although demonstrated in many fungi, aneuploidy has been best studied in *C. albicans,* where metabolism-induced oxidative stress [65] or exposure to the model host, *Caenorhabditis elegans* [66], promoted changes in ploidy and loss of heterozygosity. The high frequency of chromosome mis-segregation in *C. albicans*, and hence

the high level of adaptability of this fungal pathogen, seems to be related to the expression of a particular histone H2A variant, combined with the depletion of the centromeric histone CENP-A [67]. Of the resultant *C. albicans* aneuploids, most display reduced fitness under experimental conditions *in vitro* [68]. However, a subset of aneuploids display enhanced fitness under certain selective conditions, thereby promoting the emergence of drug resistance to azoles during prolonged antifungal therapy in patients with candidiasis [69]. In *Cryptococcus* species, large scale, reversible changes in ploidy are inducible by the host environment, which are also associated with aneuploidy. These changes underpin heterogeneity in virulence, morphotypes and drug resistance, although research in this field is still in its infancy.

Recent investigations have also revealed a role for hyper-mutating strains belonging to several important fungal pathogens that harbour an enhanced ability to rapidly evolve resistance to both biotic (for example, host) and abiotic (for example, antifungal drug) stresses, thereby providing enhanced pathogenicity and resistance respectively [70,71]. Hyper-mutating phenotypes have thus far primarily been attributed to loss of regulatory control via RNAi [70] and defects in DNA repair [71]. Further undiscovered genetic and epigenetic factors are likely to be responsible for variable rates of selection and resistance among populations of fungal pathogens.

[H1] Fungal immune evasion and manipulation

[H2] Variation in the cell wall. The fungal cell wall has a major role in immune recognition. Fungal cell walls are mainly composed of polysaccharides, some of which are not present in mammalian and other animal cells and, as such, are a rich source of pathogen-associated molecular pattern molecules (PAMPs) that are recognised by immune pattern recognition receptors (PRRs). These fungal polysaccharides include α- and β-glucans, mannans, chitin and its deacetylated form, chitosan, as well as other non-polysaccharide components such as melanin, lipids and proteins [72] (Figure 3). The amounts and types of different polysaccharides as well as their arrangement vary in different fungal genera and species. For example, α-1,3-glucans are present in *Aspergillus, Histoplasma* and *Cryptococcus* species but absent in *Candida* species [73]. The *Cryptococcal* cell wall has some considerable differences to that of most other major human fungal pathogens. For example, it contains high levels of chitosan and more β-1,6-glucans than are found in most other fungal pathogens [74]. In addition to a cell wall, *Cryptococcus* species possess an outer polysaccharide capsule (composed primarily of glucuronoxylomannan (GXM) and glucuronoxylomannogalactan (GXMGal)) that contributes to immune evasion and masking. Additionally, many fungi produce a complex, mixed morphology biofilm via the production of extracellular matrix that includes cell wall-associated components such as β-glucan.

The cell wall changes continuously in response to growth rate, cellular morphogenesis, nutrient availability and imposed stress, including immunological stress and exposure to antifungal drugs. In response to specific environmental signals associated with host niches, such as ambient pH, lactate, iron depletion or hypoxia, *C. albicans* alters the exposure of β-1,3-glucan and chitin at the cell surface [75,76]. This might represent a form of 'adaptive prediction', whereby *C. albicans* exploits host signals to activate an anticipatory response that protects against impending recognition by innate immune cells and subsequent fungal clearance [75-77], which would be consistent with coevolution of the fungus with its host. These behaviours emerge when microorganisms 'learn' to exploit one specific environmental challenge to trigger an anticipatory protective response against a second stress that is likely to follow the first. In evolutionary terms, such behaviours can be rapidly gained and lost due to their additional fitness costs [55], which explains why even closely related fungal pathogens can display different forms of adaptive prediction [78,79].

Older *C. albicans* cells have a thicker cell wall with changes in the amounts of chitin, chitooligomers, glucans and mannans, and alterations in the cell wall proteome and the way in which wall components are cross-linked [80]. In addition to the known differences between fungal hyphae and yeast or conidia [81], recent studies reported cell wall and capsule differences between *Cryptococcus species* yeast and titan cells [82] and in a range of other fungal pathogen cell types [83]. Some cell wall and capsule components (for example, *Cryptococcal* GXM and *Aspergillus* galactomannan) can also be released into the extracellular milieu. This phenotypic plasticity and heterogeneity make the cell wall a moving target for immune recognition, contributing to immune evasion and also, presumably, to the commensal lifestyles of relevant organisms [84].

PRRs have been identified for almost all carbohydrate components of fungal walls [38]. Beta-1,3 glucan , which resides in the inner cell wall layer, is a common element in the cell wall of most fungal pathogens and is recognised by several host PRRs, including dectin-1 (Ref. [85]), complement receptor 3[86,87], CD23 (Ref. [88]) and EphA2 (Ref. [89]). The other conserved element of the inner wall of most fungi is chitin, and although the exact mechanism of chitin recognition is still unclear, several receptors including Toll-like receptor 2 (TLR2), dectin-1, LysMD3, NOD2 and TLR9 have been implicated [90-92]. The outer layer of fungal cell walls is more chemically diverse, and numerous PRRs have been shown to recognise outer wall mannans, galactomannans, galactosaminogalactan, glucuronoxylo-mannans, phospholipo-mannans, α-1,4-glucan, melanin and other components. Mannans, in particular, are the ligands for several PRRs including the mannose receptor, DC-SIGN, dectin-2, MINCLE, TLR4 and galectin-3 (Ref. [93]). Interestingly, galactosaminogalactan can activate the NLRP3 inflammasome by binding to ribosomal proteins and inhibiting host cell translation [94]. Site-specific PRRs also drive fungal invasion: *Mucorales* CotH3 interacts with host nasal epithelial GRP78,

whereas CotH7 specifically targets alveolar epithelial β1 integrin, triggering EGFR and damage [38]. Fungi are thought to mask immune-stimulatory cell wall components to evade immune recognition. For example, β-1,3 glucan is thought to be masked by outer cell wall layers in several fungal pathogens, thereby evading recognition by dectin-1 (Ref. [77]). However, masking may only be effective against specific immune cell types as some immune cells, such a monocytes, have multiple receptors for outer cell wall layers (see Ref. [95] for an example). Active masking has been demonstrated in several fungi, including *H. capsulatum* and *C. albicans,* which shave extraneous superficial β-1,3 glucan from their surface using glucanases (Eng1 and Xog1, respectively) to dampen immune recognition [96,97]. In other organisms, such as *Cryptococcus gattii*, much of the chitin is deacetylated, attenuating immune recognition, which is further blocked by the presence of the outer capsule [98]. Release of the capsule polysaccharide, GXM, by *Cryptococcus* spp. can function as a protective decoy, and the presence of biofilms can physically impede the access of immune cells to the fungal cell wall [99,100].

[H2] Impact of fungal morphology on immunity. Fungal morphology shapes immunity and commensalism, and the size of the infectious propagule influences the site of infection. For example, spores of *Cryptococcus* and *Aspergillus* species (2-3μm) can penetrate deep into airways before germinating to proliferate as yeast or via invasive hyphae [101]. By contrast, *Mucorales* species spores are substantially larger (6μm to >10μm), which limits their penetration of the lower airways, leading to rhinosinusitis more frequently than pulmonary infection [102]. Yeast-phase growth of organisms such as *Candida* and *Cryptococcus spp.* is key to their haematogenous dissemination in susceptible hosts [19]. There has also been a rise of invasive and blood stream yeast infections with non-*albicans* species, *N. glabratus (C. glabrata*) as well as other species that do not form hyphae. Conversely, yeast-like gray and opaque forms of *C. albicans* are associated with a gut commensal, rather than pathogenic, lifestyle [6]. Variation in the ability of clinical isolates of *C. albicans* to filament has also been linked to the ability of these strains induce inflammation in the context of ulcerative colitis [103].

In *Candida* species, zinc limitation induces the formation of large ‘goliath’ cells, which is accompanied by hyper-adherence and increased chitin exposure [104]. *Cryptococcus species* also differentiate into large titan cells, which can be as large as 100 μm, and thus cannot easily be cleared by phagocytes [105]. The switch to titan cells is associated with production of an altered capsule and inhibition of phagocytosis of smaller fungal cells and thought to promote proliferation in the host lung. Titan cells are also associated with inducing a non-protective Th_2 tilted immune

response, which is induced through the expression of a secreted factor, Cpl1, that functions through TLR4 (Ref. [106]). *Pneumocystis* species also induce M2 polarisation of phagocytes, although the specific mechanisms driving this remain unclear [107].

[H2] Fungal evasion and modulation of phagocytic cells and their responses. Phagocyte-mediated uptake and intracellular killing of pathogens represents a major antimicrobial host mechanism, for which many fungi have developed evasion strategies (Figure 3). In addition to PAMP masking, detailed above, changes to morphology, such as titan cell formation or the generation of hyphae, can prevent uptake or allow the fungus to escape from the phagocyte through physical penetration of host cell membranes following engulfment and escape from phagosomes [12]. *C. albicans* and *N. glabratus* (*C. glabrata*) can rupture macrophages. For *C. albicans* hypha formation can physically rupture phagocytes, whereas for *N. glabratus* rupture is driven by the multiplication of yeasts to high levels within the phagosome [108]. Fungal hyphae can also induce host cell death, through pyroptosis and other cell-death mechanisms, without penetrating the host cell membranes [109]. However, hyphal growth does not always guarantee evasion, as phagocytes have the capacity to physically fold hyphae, mediating fungal clearance [110]. Phagocytes can also function as 'Trojan horses' facilitating systemic dissemination of fungi [111]. In fact, *C. neoformans* was recently shown to differentiate into 'seed cells', which are similar to the yeast form but with an altered surface, which enhances their uptake by macrophages and promotes extrapulmonary dissemination [112].

Other phagocytes, such as neutrophils also have a key role through the phagocytosis and killing of fungi. Moreover, neutrophils can undergo a programmed cell death response to form neutrophil extracellular traps (NETs) that can entangle, immobilize and kill fungi. Notably, the formation of NETs is tightly regulated by pathogen size and primarily induced following encounter with non-ingestible fungal forms, such as hyphae [113]. However, fungi have developed mechanisms to evade NETosis through, for example, the formation of biofilms, changes in fungal cell wall components and the secretion of NET-degrading enzymes, such as DNAse [114].

Many fungi have also developed mechanisms that enable them to survive within host cells, using strategies including inhibition of phagosome maturation, resisting degradation within the phagolysosome, escape from the phagosome or modulation of host metabolism. Following uptake, *C. neoformans*, for example, can prevent acidification, calcium flux and protease activity within the phagosome, and limit phagolysosome maturation [115]. *C. neoformans* can also induce non-lytic expulsion (vomocytosis) to escape the host cell [116,117]. This process has also been described for other fungal pathogens including *Candida* spp.[118], and been linked to fungal cell transfer between phagocytes [119]. Another

example of an intracellular survival mechanism is the secreted HscA protein of *A. fumigatus*, which diverts the phagosomal sorting process in epithelial cells to facilitate fungal persistence and expulsion via the endosomal recycling pathway [120]. Other pathogens also secrete virulence factors that modulate host cell function, such as *H. capsulatum* Cbp1, which enables escape by killing the host cell [27]. By contrast, *C. auris* kills host macrophages by inducing immunometabolic reprogramming, resulting in host-cell metabolic stress induced through glucose starvation [121].

Perhaps uniquely among human fungal pathogens, *Pneumocystis jirovecii* survives in close proximity to host cells. These pathogens exist as extracellular, obligate biotroph organisms that bind type I and type II pneumocytes in the lung and avoid immune detection in this niche [122]. Although not completely understood, in part due to the inability to culture this organism *in vitro*, there is evidence that indicates that the trophic form (a very simple life stage form with no cell wall, β-glucan or chitin) alters dendritic cell function and their ability to induce adaptive immunity, thereby enabling fungal survival [123].

Some pathogenic fungi, such as *Cryptococcus* species, may be sequestered by the host within tissue granulomas that are formed by the host to control infection and prevent dissemination. However, if the immune system becomes weakened, the fungi can escape to cause disease. For example, patients with multiple sclerosis that undergo long-term treatment with FTY720 (a sphingosine-1-phosphate receptor antagonist that affects immune cell function) are prone to develop *C. neoformans* infections [124]. In mouse models, FTY720 treatment led to disorganization of granulomas and resurgence of cryptococcosis [125].

Many fungi secrete factors including polysaccharides, toxins, small metabolites and secreted proteins that prevent phagocyte uptake, dampen immune cell activation and/or alter immune responses. For example, swollen *Mucorales* spores can evade uptake by secreting antiphagocytic factors of fungal origin, such as mucoricin, or factors derived from bacterial endosymbionts such as rhizoxin-producing *Mycetohabitans rhizoxinica* [126,127]. In addition to Cpl1, described above, *C. neoformans* sheds capsular polysaccharides (including GXM) that possess considerable immunoregulatory activities, including suppression of inflammatory cell recruitment and the development of adaptive immunity [128]. *C. neoformans* also secretes small metabolites, such as DL-p-hydroxyphenyllactic acid (HPLA) and DL-indole- 3-lactic acid (ILA) that inhibit secretion of key cytokines, such as IL-1β [36]. Another example of a secreted immune-modulatory fungal factor is Pra1, which is involved in zinc acquisition in *C. albicans* [129]. Pra1, which is induced by low levels of zinc, interacts with multiple components of the complement pathway, facilitating immune evasion [130], and binds to $CD4^+$ T cells, which affects their proliferation and cytokine production [131]. The *A. fumigatus* orthologue of Pra1 (named AspF2) has a similar impact on immunity, which suggests conservation of the immune-modulating function of this protein across

different pathogenic fungal species [132]. Intriguingly, zinc-regulated *C. albicans* Pra1 is also a potent neutrophil chemoattractant, which promotes fungal clearance during systemic infection [133] but exacerbates inflammation in vulvovaginal candidiasis (VVC) [134]. Zinc supplementation, to downregulate the levels of Pra1, offers therapeutic benefit to women suffering from recurrent VVC [134].

[H1] The host and clinical perspective

[H2] Host immunity. Rapid advances in knowledge of antifungal immune mechanisms [reviewed in Ref. [38]] has contributed considerably to our understanding of fungal pathobiology. Discoveries of the importance and underlying components of innate and adaptive Th1 (required for the control of systemic infections) and Th17 (required for the control of mucosal infections) immunity have not only provided insights to the susceptibility of specific patient groups to fungal infection but have also offered therapeutic prospects (detailed below). For example, chronic mucocutaneous candidiasis (CMC) is a common consequence of numerous inborn errors of the immune system (such as autosomal dominant hyper IgE syndrome) that involve alterations in Th17 immunity [135]. However, the functions of Th1 versus Th17 immunity are likely to be oversimplified as susceptibility to at least one CMC-related disease (autoimmune polyendocrinopathy–candidiasis–ectodermal dystrophy (APECED)) was also linked to aberrant Th1 immunity [136]. Far less is understood about the contributions of other key adaptive immune components, including Treg and Th2 responses, with the former thought to have a role in limiting pathology and the latter linked to non-protective and allergic responses [137].

Substantial advances have also been made in understanding the contributions of individual PRRs, intracellular signalling pathways, specific immune cell populations, immune modulators (such as cytokines and chemokines) and host cell metabolism during fungal infections, as well as their roles in shaping tissue-specific immunity (for reviews, see for example Refs. [38,138,139]). For example, in the kidney fungal sensing by dectin-1 causes neutrophils to undergo a metabolic shift by upregulating glucose uptake through glucose transporter-1, which enhances their fungicidal functions, including the production of ROS and NET formation [140]. Recent discoveries have also started to reveal how the immune system can influence the balance of pathogenicity versus commensalism in relevant species. For example, in the gastrointestinal tract a Paneth cell antimicrobial peptide (peptide YY) as well as hyphal-specific IgA supress the formation of the tissue damaging *C. albicans* hyphal morphotype, favouring the yeast form and commensalism [141-143].

Optimal activation of the host immune response is essential for controlling fungal infections, but balance is essential to avoid excessive or prolonged inflammation, which can have severe pathological consequences and may be a cause of mortality. This is exemplified by immune reconstitution inflammatory syndrome (IRIS), a paradoxical hyper-inflammatory response that occurs in immunosuppressed patients with a pre-existing fungal infection when their immune function is restored. Immunosuppressed individuals living with HIV/AIDS are at risk of developing cryptococcal IRIS following initiation of antiretroviral therapy, and IRIS can occur with other fungal diseases including histoplasmosis [144] and *P. jirovecii* pneumonia [145]. In the context of mouse oropharyngeal candidiasis, fungal secretion of candidalysin and activation of the epithelium (via EGFR -mediated pathways) is essential for driving host damage responses and orchestrating sterilising immunity [146]. This is not just limited to the oral barrier, as candidalysin also forms a key signal for microglial-mediated clearance of *C. albicans* cerebral mycosis in mice [147]. Fungi can also modify human macrophage function via boosting arginase expression [148], which in other disease settings is linked to wound repair [149]. However, how fungi modify host repair mechanisms is still poorly understood.

[H2] Fungal exacerbation and regulation of disease. It is becoming increasingly clear that fungi have an enormous influence on human physiology, although the underlying mechanisms are still largely unknown. Although commensal fungi can have homeostatic roles in promoting beneficial immune responses [150], recent discoveries have also begun to reveal the impact of both commensal fungi (see for example Ref. [151]) and pathogenic fungi on the immune pathology of cancer as well as inflammatory and autoimmune diseases such as inflammatory bowel disease and alcoholic liver disease (reviewed in Refs. [152,153]). Indeed, changes to the mycobiome can have a considerable impact on clinical outcomes. For example, in patients that undergo allogeneic haematopoietic stem-cell transplantation, pre-engraftment dominance of *C. parapsilosis* (which accounts for >90% of fungi detected by *ITS1* sequencing in stool samples and an associated loss in bacterial diversity in this study) correlated with reduced overall survival and increased transplant-associated mortality [154].

Fungi are thought to be major drivers of allergic disease with estimates that over half of the individuals with asthma (~150 million sufferers globally) display sensitivity to fungi [155]. Moreover, individuals with chronic lung diseases, including patients with asthma, bronchiectasis, cystic fibrosis and chronic obstructive pulmonary disease (COPD), show increased fungal airway colonisation and infection associated with increased disease severity [156-158]. Fungi are likely to exacerbate allergy partly via multiple secreted factors including proteases and toxins. For example, *Alternaria* spp. proteases damage the lung epithelial cell lining to release IL-33, which results in innate lymphoid cells co-ordinated allergic

inflammation [159], whereas *Aspergillus* spp. proteases can activate calcium channels (TRPV4) on epithelial club cells triggering $CD4^+$ T cell-dependent recruitment of eosinophils[160]. By contrast, the fungal toxin candidalysin activates platelets to exacerbate allergic inflammatory $CD4^+$ T cells [161]. The precise mechanisms through which T cells are activated are likely to be a critical determinant of allergic diseases, and functionally distinct populations of effector Th cells targeting different *A. fumigatus* proteins were recently detected in patients with cystic fibrosis [162].

Fungi also exacerbate the pathology of viral and bacterial infections, including tuberculosis [163,164]. In fact, interactions between bacteria and fungi can directly influence fungal virulence (Box 2). For respiratory viral infections, in particular, there is now considerable evidence that fungal co-infections are associated with higher mortality rates (excess mortality of 50% compared to those without fungal coinfection). For example, influenza-associated pulmonary aspergillosis (IAPA) during the 2009 H1N1 pandemic [165] and COVID-19 associated pulmonary aspergillosis (CAPA) during the COVID-19 pandemic [166] have revealed the susceptibility of patients with severe viral pneumonia to develop invasive pulmonary fungal disease. Although the pathophysiology of viral–fungal coinfections is unclear, disruption of the respiratory barrier and impaired antifungal immune responses resulting from viral infection have been proposed to have a role (reviewed in Ref. [167]).

[H2] Invasive fungal diseases. Diagnosis of patients who are at risk of developing invasive fungal disease (IFD) is paramount as clinical signs and symptoms are often non-specific and can be comparable to those seen in invasive bacterial disease [168] (Table 1 and Supplementary Table 1). Except for endemic dimorphic fungi, invasive fungal pathogens are opportunistic in nature, causing disease in those with a compromised immune system (including primary immune deficiencies), severely ill patients admitted to an intensive care unit, among patients with invasive medical interventions such as abdominal surgery, and those with predisposing conditions including chronic lung diseases and diabetes mellitus. Endemic dimorphic mycoses affect both immunocompetent and immunocompromised patients, but those with underlying immunosuppression usually display more severe disease phenotypes [169]. IFD caused by moulds, such as *Aspergillus* spp. and the *Mucorales*, are mainly localised in the sinus or lungs (but can disseminate) [170,171], *P. jirovecii* infection is primarily pulmonary [172], whereas IFD caused by yeasts, for example, *Candida* spp., and moulds in the genus *Fusarium* predominantly result in a bloodstream infection, with or without dissemination to other organs [173,174]. *C. neoformans* has a predilection for the central nervous system (neurotropic) and infection will result in cryptococcal meningoencephalitis in most cases, in particular in HIV-infected individuals with advanced defects in cellular immunity [175].

The case fatality of IFD in immunocompromised patients is high (ranging from 30% to 80%), especially if there is no recovery of the underlying immunocompromised state, and if there is disseminated disease. The high case : fatality ratio is one of the reasons that empiric antifungal therapy is frequently started when an IFD is suspected, based on the underlying condition and risk factors (for example, prolonged febrile neutropenia) with no response to antibacterial therapy. Fungal biomarkers and PCR assays to detect *Aspergillus*, *Candida*, *Cryptococcus* species, mucoraceous moulds and *P. jirovecii* can guide the decision-making process to start or stop antifungal therapy, but positive cultures from otherwise sterile material and/or direct microscopy or histopathology on tissue samples is required to make a definitive diagnosis. Over the past decade, a number of international management guidelines addressing specific IFD have been developed, providing a useful tool in guiding management decisions at the bedside [174,176-178]. Nevertheless, patient care is substantially compromised by difficulties in diagnosis, a limited arsenal of anti-fungal agents and the rapid rise of anti-fungal drug resistance (described below) (Table 2).

[H2] Host-directed therapy. Our increased understanding of the complex interplay between fungi and host immune responses, and their contributions to disease pathology and the specific pathways involved, underpins the increasing clinical use of host-directed therapies. In the setting of overactive or dysfunctional immune responses, adjunctive corticosteroids, for example, are used to treat allergic fungal diseases, severe *Pneumocystis* pneumonia, and HIV- and non-HIV-associated immune reconstitution reactions in the setting of fungal infection [179]. The more specific agents for fungal allergy, omalizumab, mepolizumab and dupilumab, which target IgE, IL-5 and IL-4, respectively, show promise in allergic bronchopulmonary aspergillosis [180-182]. Cytokines, notably IFN-γ and GM-CSF, have been successfully used to promote Th1 immunity and activate neutrophils and mononuclear phagocytes in several infectious settings, including aspergillosis in patients with chronic granulomatous disease, HIV-associated cryptococcal meningitis and to prevent and manage fungal infections in allogeneic bone marrow transplant recipients [183]. A randomized trial of adjunctive IFN-γ in candidaemia has recently started (NCT04979052). Selection and transfer of donor *Aspergillus*-specific T cells to haploidentical transplant recipients reduced *Aspergillus*-related deaths [184], and protocols for generating fungal specific T cells have subsequently been improved [185].

Our growing knowledge of the function of fungal PRRs is also offering new therapeutic avenues. For example, topical administration of a TLR agonist, imiquimod, markedly improves chromoblastomycosis lesions [186] (a chronic fungal infection of the skin), whereas coating amphotericin B liposomes with the binding domain of dectin-2 substantially increased antifungal efficacy in a mouse model of pulmonary

aspergillosis [187]. PRRs are also being considered for chimeric antigen receptor (CAR) T cell therapies, such as CAR T-cells expressing dectin-1 fused to CD28 and CD3ζ signalling domains, which inhibited *Aspergillus* growth *in vitro* and *in vivo* [188].

Disappointingly, there is still no vaccine in clinical use for any human fungal disease. However, a few are in development, including an agglutinin-like sequence 3 (Als3)-based *Candida albicans* vaccine (NDV-3), which showed benefit in a randomized trial with women with recurrent VVC [189]. Other strategies, including delivery of antigens within glucan or glucan/chitin particles have potential for the future (reviewed in Ref. [190]).

[H1] Conclusions

The number of humans succumbing to fungal infections are projected to increase, due to a rapid rise in resistance to antifungal drugs, developments in modern medicine that alter protective immunity, and the emergence of new pathogens, including zoonoses. Until recently, fungal antimicrobial resistance has received much less attention than the threat of drug resistance in other pathogens. However, highly drug-resistant species such as *C. auris,* environmentally (predominantly agricultural) driven increases in resistance to azoles in *A. fumigatus* [191] and the occurrence of fungal infections in newly recognised patient populations with SARS-CoV-2 and influenza infections have heightened concerns about the increasing difficulties in treating natural or acquired resistance in fungal species [191,192]. Several fungi, including azole-resistant *A. fumigatus*, *N. glabratus*, *P. kudriavzevii*, *C. auris*, *L. prolificans* and the Mucorales, now present major clinical challenges to the effectiveness of currently available antifungals [191].

Clinically, the consequences of fungal antimicrobial resistance are made worse by inadequate and late diagnosis, and the lack of availability and accessibility of antifungal options in impoverished regions of the world that have high burdens of IFDs. Improved agents, formulations and novel classes of antifungals, such as rezafungin, encochleated amphotericin B, opelconazole, ibrexafungerp, olorofim and fosmanogepix, offer new clinical opportunities [193]. In addition, an exploration of the benefits of combination therapy [194] and careful antifungal drug stewardship [195] are critical to extend the durability and efficacy of the antifungal pipeline in the context of growing levels of fungal AMR.

The incidence and burden of fungal infections are also increasing and evolving because of medical advances. In addition to rising numbers of immunosuppressed patients, particularly those undergoing therapy for cancer or transplantation, increasing use of monoclonal antibodies and kinase inhibitors with specific immunological targets for a wide range of autoimmune and inflammatory diseases is resulting in

new patient groups at heightened risk of fungal infections (reviewed in Ref. [38]). In addition, international travel and trade, pollution and population expansion within areas endemic for geographically restricted dimorphic fungi, has further increased the numbers of persons at risk.

The emergence of new pathogens, driven by, or coinciding with, climate change, poses considerable challenges for the future. Genomic studies suggest a near-simultaneous emergence of *C. auris* on multiple continents [196,197]. Another example is *Emergomyces africanus*, (previously *Emmonsia* species), the first new thermally dimorphic fungal pathogen identified in nearly 60 years, found originally in South Africa with closely related species identified on four continents [198,199]. Furthermore, sporotrichosis caused by *Sporothrix brasiliensis*, which is transmitted by cats, represents the first reported invasive fungal zoonotic disease. Originally considered endemic to Brazil, cat-transmitted sporotrichosis is now spreading throughout South America and cases are now being reported across the world [200].

In closing, our knowledge of human fungal infections has advanced considerably. Tackling the extant and emerging threats posed by these pathogens will require continued and greater awareness and more investment from governments, funding agencies and industry. Increased capacity in this field is essential to tackle the huge burden of human fungal diseases, including the billions of people suffering non-life-threatening infections by dermatophytes (including drug-resistant pandemic species such as *Trichophyton indotineae* [201]*)* which were not covered in this Review. As we highlighted over a decade ago [202],we must continue to gain a better understanding of fungal pathobiology to aid the development of safer and more effective antifungal management strategies in the future.

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Author contributions

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Table 1. Diagnostic considerations and antifungal resistance.

	Diagnostic							Resistance					
Fungal disease	**Micro-scopy and culture**	**Biomarker: BDG**	**Biomarker: GM**	**Molecular test: Sp-sp PCR**	**Molecular test: Pan fungal PCR***	**Antigen**	**Antibody**	**Fluconazole**	**Ampho-tericin B**	**mould-active azoles**	**Echino-candins**	**Flucyto-sine**	**Terbinafine**
Aspergillosis	BALf, T, sBF, Sp	B, CSF	B, BALf, CSF	B, BALf, T, sBF	B, T, sBF	GM only: B, BALf, CSF	B#	IR	Very rare (H,IR, AR), *A. terreus* (IR)	AR (environment, l-Th)	Rare (IR, AR)	IR[$]	NR
Candidosis	B, T, CSF, sBF	B, CSF	ND	B, T, sBF	B, T, sBF	B, CSF	B, CSF	Sp-sp (IR) l-Th (AR)	Sp-sp (IR)	Sp-sp (IR), l-Th (AR)	Sp-sp (IR), l-Th (AR; rare)	Sp-sp (IR), l-Th (AR)	NR
Cryptococcosis	CSF, B	ND	ND	X	CSF, B	CSF B	X	H, AR	Rare	H, AR (both not common)	IR	H, AR	NR
Other yeast species	B, T, CSF, sBF	B, CSF	ND	X	B, T, sBF	X	X	Sp-sp (IR) l-Th (AR)	Sp-sp (IR)	Sp-sp (IR), l-Th (AR)	Sp-sp (IR), l-Th (AR; rare)	Sp-sp (IR), l-Th (AR)	NR
Pneumocystis	BALf, Sp (microscopy only)	B, BALf	ND	BALf, B	BALf, B	X	X	IR	IR	IR	RS (active against cyst form)	IR	NR
Mucoro-mycosis	BALf, T, sBF	ND	ND	B, T	B, T	X	X	IR	Very rare	IR (exception posa and isa for some species)	IR	IR	NR
Hyalophyphomycosis													
Fusarium	T, CSF, sBF, Sp	B, CSF	B, BALf, CSF	X	B, T, sBF	X	X	IR	IR (common)	IR encountered (vori treatment of choice)	IR	IR[$]	NR
Scedosporium	T, CSF, sBF, Sp	B, CSF	ND	X	B, T, sBF	X	X	IR	IR (common)	Rare	IR	IR	NR
Lomentospora	T, CSF, sBF, Sp	B, CSF	ND	X	B, T, sBF	X	X	IR	IR	IR	IR	IR	NR
Phaeohyphomycosis													

Cladophialo-phora	T, CSF, sBF, Sp	B, CSF	ND	X	B, T, sBF	X	X	IR	Rare	Rare	IR	IR[$]	NR
Exophiala	T, CSF, sBF, Sp	B, CSF	ND	X	B, T, sBF	X	X	IR	Rare	AR (rare)	IR	IR[$]	NR
Rhinocladiella	T, CSF, sBF, Sp	B, CSF	ND	X	B, T, sBF	X	X	IR	IR[$]	Rare	IR	IR[$]	NR
Endemic dimorphic mycoses													
Blastomycosis	T, CSF, sBF, Sp	ND	ND	X	B, T, sBF, BALf	X	B	IR	Rare	Rare	RS, IR	NR	NR
Coccidioidomycosis	T, CSF, sBF, Sp	P	CR[&]	X	B, T, sBF, BALf	X	B	Rare	Rare	Rare	RS, IR	NR	NR
Emergomycosis	T, CSF, sBF, Sp	P	CR[&]	X	B, T, sBF, BALf	X	B	IR	Rare	Rare	RS, IR	NR	NR
Histoplasmosis	B, T, CSF, sBF (BM), Sp	P	CR[&]	X	B, T, sBF, BALf	B, U	B	Rare	Rare	Rare	RS, IR	NR	NR
Paracoccidioidomycosis	T, CSF, sBF, Sp	P	CR[&]	X	B, T, sBF, BALf	X	B	Rare	Rare	Rare	RS, IR	NR	NR
Talaromycosis	T, CSF, sBF, Sp	P	CR[&]	X	B, T, sBF, BALf	X	B	IR	Rare	Rare	RS, IR	NR	NR
Superficial and subcutaneous													
Alternaria	T	ND	ND	X	T	X	X	IR	Rare	IR (voriconazole)	RS, IR	IR[$]	NR
Candida	Swab, T	ND	ND	T	T	X	X	Sp-sp (IR) l-Th (AR)	Sp-sp (IR)	Sp-sp (IR), l-Th (AR)	Sp-sp (IR), l-Th (AR; rare)	Sp-sp (IR), l-Th (AR)	NR
Chromoblasto-mycosis	T	ND	ND	X	T	X	X	IR	Rare	Uncommon	RS, IR	IR[$]	NR
Dermato-phytosis	T	ND	ND	T	T	X	X	Sp-sp (IR) l-Th (AR)	Rare	Sp-sp (IR), l-Th (AR)	RS, IR	IR	rare (IR and AR) except *T. indotineae, M. canis* (AR)
Eumycetoma	T	ND	ND	X	T	X	X	IR	Rare	Uncommon	RS, IR	IR[$]	NR
Sporotrichosis	T	ND	CR[&]	X	T	X	X	IR	IR common	IR common	IR	IR[$]	NR

BALf: bronchoalveolar lavage fluid, T; tissue, sBF: sterile body fluids, Sp: sputum, B: blood, CSF: cerebrospinal fluid, BM: bone marrow, U: urine, BDG: beta-D-glucan, GM: galactomannan, P: present in most strains, X: unavailable commercially, although in-house methods may be available, ND: not detected or only at very low levels, CR: cross-reactivity, Sp-sp: species-specific, l-Th: long-term therapy, isa: isavuconazole,

posa: posaconazole, vori: voriconazole, H: heterogenous; IR: intrinsic resistance; RS: reduced susceptibility; AR: acquired resistance; NR: not relevant

[&] May have clinical utility.

* Panfungal PCR can be used on BALf but it is likely to detect only the predominant organism and commensal yeast are often detected. It is most helpful on BALf for pneumocystis or the endemic dimorphic mycoses.

In immunocompetent patients (for example, aspergilloma, endocarditis).

[$] May be helpful in combination.

Table 2. Unmet clinical needs for systemic fungal infections*

Needs of progress and/or promising leads	Continued need and/or stage of development	
Fungal vaccines	*Candida albicans* vaccine (NDV-3) in phase I clinical trial, showing modest benefit in RVVC	As yet no licensed fungal vaccine for humans
Candida auris infection control in ICU	Guidelines have been developed [203]; Examples of best practice have been published [204-206]	Wider surveillance and implementation are required
Global surveillance	The WHO Global antimicrobial resistance surveillance system (GLASS) now includes *Candida* spp.	Much more work is needed in regard to the optimization and harmonization of fungal surveillance
Better rapid, point-of-care diagnostics, enabling earlier, targeted treatment	Cryptococcal Ag LFA [in use] *Histoplasma* Ag EIA and LFA format [in use] *Talaromyces* Ag detection (not yet commercialized) [in development] *Mucorales* Ag LFA format (preclinical)	Further improvement in diagnostics for invasive aspergillosis, invasive mould infection and candidiasis (with sensitivity data, species identification) are needed to enable a shift from empiric towards targeted therapy *Pneumocystis* diagnostics that suitable for LMIC are needed General accessibility to antifungals and fungal diagnostics is a continued need in LMIC

Improved antifungal stewardship	Programmes and guidelines have been developed	Implementation studies to drive uptake, and measure impact Better diagnostics
New antifungals, new classes Selected for efficacy, safety, spectrum (including against resistant isolates), cidal (compromised hosts), oral and intravenous formulation, high barrier to resistance development, long-acting, synergetic potential with existing agents, and ideally with related compounds not developed for agricultural use	Ibrexafungerp (activity: *Candida* spp.) oral β-glucan synthase inhibitor Rezafungin, long-acting, once-weekly echinocandin Olorofim (activity: *Aspergillus* spp., rare moulds, Coccidioides app.) inhibits pyrimidine synthesis Fosmanogepix APX001 (activity: *Candida* spp., *Aspergillus* spp.) inhibits GPI-anchored wall transfer protein 1 (Gwt1) APX-2039, enhanced activity against *Cryptococcu*s Sfunga-001, AmB derivative, based on concept of sterol sponge mechanism of action Nebulized opelconazole (PC945) (Pulmocide)	Licensed for rVVC Licensed for candidaemia, invasive candidiasis In phase 3 for IA, vs L-AmB Small phase 2 candidaemia trials completed Pre-clinical stage Pre-clinical stage In phase 3 trial for invasive aspergillosis in addition to systemic SOC
Optimized or extended use of existing antifungals Combinations (synergy and/or resistance protection), and optimized PK/PD	Dectin-2 coated AmB-liposomes Cryptococcal meningitis induction regimens Azole echinocandin combination trial for invasive aspergillosis	Pre-clinical Phase II/III clinical trials Phase III clinical trials

		Ibrexafungerp in phase 2 trial in combination with voriconazole versus voriconazole alone in invasive aspergillosis Rezafungin in combination with 7 days co-trimoxazole versus co-trimoxazole alone in phase 2 trial in PCP
Global antifungal drug access	5FC, two new manufacturers, cost have halved since 2018; 5FC and L-AmB provision through Global Fund for cryptococcal meningitis; generic voriconazole, echinocandins	Only one main manufacturer of L-AmB
Anti-virulence agents	Targeting filamentation in *Candida albicans,* for example: filastatin, compound 61894700 MAb against mucoricin, CotH3, for mucormycosis	Pre-clinical Pre-clinical
Host-directed approaches, immune-diagnostics to enable patient-specific targeting	MAbs targeting IgE, IL-5, IL-4 for allergic fungal diseases Cytokines (IFN–γ, GM-CSF) Engineered β-glucan specific CAR-T cells	Evidence for immunotherapies based mainly on small case series There is a need for more randomized controlled trials, and rapid diagnostics for identifying immune defects
Novel Clinical Trial Strategies and Designs	Adaptive designs, co-ordinated funding and implementation and streamlined approval processes delivered rapid large trials in COVID-19	Novel, co-operative approaches are needed to facilitate trials in systemic fungal infection

LFA lateral flow assay; EIA enzyme immune assays; LMIC low and middle-income countries; RVVC recurrent vulvovaginal candidiasis; SOC standard of care; L-AMB liposomal amphotericin B; PK/PD pharmacokinetics/pharmacodynamics;; 5FC flucytosine; PCP pneumocystis pneumonia; MAb monoclonal antibody; IFN-γ interferon gamma; GM-CSF granulocyte macrophage colony stimulating factor; CAR-T chimeric antigen receptor

*This is broad overview of priorities and not exhaustive. Many aspects are not highlighted, including the unmet needs associated with non-invasive fungal diseases.

Figure 1. Human pathogenic fungi designated as critical, high and medium risk by the World Health Organisation. Shown are 18 fungal pathogens that have been categorized as critical, high or medium risk to human health by the WHO during compilation of the first Fungal Pathogens Priority List (FPPL) [2]. The FPPL was generated via multicriteria decision analysis to achieve a systematic prioritisation of fungal pathogens causing acute or subacute invasive disease, with a view to identifying urgent unmet research and development needs, areas for sustainable investment and to inform and enable policymakers. Ten assessment criteria were used for ranking including incidence, case fatality rates, global distribution, antifungal resistance and access to diagnostics and treatments. Primary niches of the fungi are designated as green circles for pathogens of environmental origin (for example, soil or airborne fungi) or orange circles for pathogens derived from human commensal mycobiota). Fungal pathogens acquired from environmental sources may enter the human body (bottom left) to cause disease via inhalation (1 and 2) or traumatic implantation (3 and 4), including during surgery. Human commensals may translocate from the skin (5) and/or mucosa (6 and 7) to cause disease or via traumatic implantation (7) including during surgery. At-risk populations, routes of acquisition and corresponding fungal diseases are documented in supplementary Table 1. Drawings are indicative of fungal morphologies and are not to scale.

Figure 2: Fungal virulence factors and virulence attributes.

Pathogen potential in diverse fungi emerges through common phenotypic traits that enable growth in mammalian host tissues. Pathogenesis has emerged repeatedly and independently across the fungal kingdom. Representative species and their key traits are shown**. Pathogen potential in mammals** is underpinned by **adaptation to host environment conditions**, including temperature, pH, CO_2 and nutrient acquisition. Close association with humans can occur either as commensal (*Candida* spp. with *C. albicans* shown as a representative member) or obligate (*Pneumocystis*) interactions, with metabolic rewiring enabling (*Candida)* or restricting (*Pneumocysistis*) host niche adaptation. **Drivers of pathogenesis** include morphological changes, secreted factors and host-specific strategies. **Morphogenesis:** the germination of spores and the switch to invasive specific growth forms characterize pathogenesis of *Aspergillus, Fusarium,* and *Mucorales* species. Thermal dimorphism across a wide range of species supports morphological switches that enable dissemination or tissue penetration and are

associated with cell wall changes (see, Figure 3) relevant to stress resistance and immune evasion. Pleomorphic growth forms (*Cryptococcus; Candida*) enable dissemination, translocation across tissue barriers and tissue invasion. **Secreted factors:** the expression of cell surface adhesins is a major driver of environmental spread (*C. auris* Scf1) and adhesion to host cells (*Rhizopus* CotH via Gpr78; *Blastomyces* BAD1 via CR3; *Coccidioides* SOWgp via TLR-2 and Dectin-1; *Candida* Als3 via E- or N-cadherin; *Candida* Hwp1 via transglutaminase), and these can additionally be degraded by fungal enzymes to enable masking (*Coccidioides* Mep1). Secreted proteins can additionally modulate host immune factors (*Blastomyces* BAD1, *Cryptococcus* Cpl1) or detoxify host responses (*Candida* Sod5, ROS). In addition, micronutrient acquisition is mediated via secreted factors including siderophores or zincophores (Pra1), iron-binding receptors (Als3), or scavenging via xenosiderophores. Secreted factors can also directly modulate host interaction including *A. fumigatus* Mep1 and *Cryptococcus* App1 that cleave complement components (C3, C4 and C5) to block phagocyte recruitment, *Blastomyces* DppIVA that targets host cytokines and chemokines (CXC) , and calcium-binding protein 1 (CBP1) that promotes phagocyte cell death in *Histoplasma capsulatum* and *Paracoccidiodes spp*. Proteases (*Cryptococcus* Mpr1), hydrolytic enzymes (*Candida* Sap4-6), and toxins (*Rhizopus* mucoricin; *Candida;* candidalysin) can directly impact tissue barriers. Translocation across tissue barriers can be mediated by host cells (Trojan horse model) or via the formation of trans-cellular tunnels. Sizes of the fungal pathogens drawn are not to scale. EV, extracellular vesicle.

Figure 3. Fungal immune evasion mechanisms. Fungal immune evasion strategies include differing chemistries of the outer cell wall (a); modification of the cell wall associated with the yeast–hypha transition of *Candida albicans* (b) and a range of encounters and interactions with phagocytes (c). a) The inner cell wall of these fungi (green) contains β-1,3 glucan (which is a strong immune agonist) and a variety of other polysaccharides in different fungi which are not represented in this diagram. Immune recognition of components of the inner cell wall can be blocked or shielded by the presence of a gelatinous capsule of *Cryptococcus neoformans* that also generates sloughed decoy capsule fragments; the shaving of superficial β-1,3 glucan by the *Candida albicans* glucanase Xog1; the presence of an immunologically inert outer layer of rodlets of the *Aspergillus* conidium; or α-1,3 glucan in the outer wall of *Histoplasma capsulatum* and *Blastomyces dermatitidis* that shields the underlying β-1,3 glucan from detection. b) Model of the cell wall of *C. albicans* of the yeast and hyphal form showing greatly

increased chitin content of hyphae and differences in the outer cell wall mannan chemistry (indicated by the purple structures). These lead for example, to increased recognition of hyphae by the mannose receptor immune receptor. c) Interactions of *C. neoformans* or *C. albicans* with macrophages, exemplifying strategies used by a wider range of fungal pathogens. Cellular gigantism is illustrated by a Titan cell of *C. neoformans* (or Goliath cells of *C. albicans* or spherule formation by *Coccidioides immitis*) that are too large to be easily engulfed. The use of secreted decoy molecules such as capsule fragments of *C. neoformans* that block macrophage function or Bad1 production by *B. dermatiditis* that interferes with macrophage and neutrophil-function. Non-lytic expulsion (vomocytosis) of a yeast cell of *C. neoformans* and other unicellular pathogens. Induced hypha formation and piecing of the macrophage membrane by a *C. albicans* hypha and the induction of pyroptosis mediated macrophage cell death. Formation of a 'frustrated' phagosome with a collar of formin-dependent actin enabling the partial engulfment and inhibition of long hyphae that cannot be completely phagocytosed. The active folding of a long hypha of *C. albicans* within the phagosome of a macrophage to enable its destruction. The modification of the toxic phagolysosomal environment by *C. albicans* by affecting RAB GTPase localisation, and restricting phagosome acidification, oxidative or nitrosative damage. The induction of the pore forming toxin candidalysin or the fungus activates the NLRP3 inflammasome and the release of Gasdermin D, which leads to phagocyte membrane leakage.

Part b is adapted with permission from Gow, Lenardon et al., 2022 [207].

Box 1: Emergence of fungal antimicrobial resistance and new pathogens.

It is critical to make a concerted effort to understand the lifecycle and reservoirs of human fungal pathogens, and to complement this with strict antimicrobial resistance (AMR) surveillance and improved diagnosis. The high incidence of fungal diseases in susceptible individuals indicates that humans are frequently exposed to fungal pathogens. Moreover, the worldwide dissemination of previously endemic pathogens, including multi-drug resistant *Candida auris* [5], suggests that fungal diseases are spreading and incidence of drug resistance is rising.

[bH1] Overuse of pharmaceuticals drive AMR in fungi. In 2006, to prevent AMR in bacterial diseases, the EU restricted agricultural and farm use of antibiotics, with further restrictions enacted in 2022 (Ref. [208]). Unfortunately, these regulations do not apply to antifungals. Thus, the extensive use of azoles in agriculture has already been linked to the rise in infections by azole-resistant *Aspergillus fumigatus* [209]. Another

example is *Trichophyton indontineae* which is intrinsically resistant to terbinafine and its emergence has been attributed to misuse and overuse of topical antifungals and corticosteroids [210].

[bH1] Anthropogenic activity driving the emergence of new pathogens. Fungi typically thrive in moderate temperatures, with only a fraction of fungi tolerating 37°C. High basal internal temperature of mammals provides a key innate resistance to fungi. These fungal–mammal interactions may have contributed to the evolution of thermotolerance of mammals [197,211]. A key example is white nose syndrome (WNS), a fungal infection in hibernating bats that occurs when their body temperature drops, resulting in substantially reduced bat populations. Worldwide global warming reduces this thermal barrier and may be driving the adaptation of 'thermosensitive' fungi to mammalian body temperatures. Indeed, an entomopathogenic fungus, *Metarhizium anisopliae*, was rapidly adapted to higher temperatures by laboratory thermal selection [212]**.** Urban heat traps may compound this risk: fungal species in cities may become more thermotolerant than their rural counterparts.

Human travel and globalization also facilitate the dissemination of pathogens. WNS, as an example, is thought to have spread to the USA from endemic populations in Europe, possibly on cavers' equipment. Moreover, the global spread of chytrids, causing dozens of extinctions in amphibians, is attributed to global trade in amphibians [213]**.**

BOX 2: Impact of fungal–bacterial interactions on virulence.

Fungal pathogens exist in the wider context of the microbiota exhibiting both antagonistic and agonistic interactions with bacteria that impact fungal virulence across important host sites. The presence or absence of a particular fungal species can influence the abundance of bacterial taxa, and bacteria can in turn influence fungal growth, morphogenesis and virulence (reviewed in Refs. [214-216]). Interactions can be species-specific, mediated by the expression of secreted factors or surface-expressed molecules [217-220], via cross-utilization of metabolic biproducts, secondary metabolites, or micronutrient depletion [221-224], or via interactions between fungal and bacterial microbe-associated molecular patterns (MAMPs) such as cell wall sugars, which can influence fungal morphology, growth and virulence [30,225-229] . Various bacterial effectors can also directly inhibit fungal growth via type VI secretion systems [230]. Factors shed or secreted by bacteria can also indirectly affect fungal morphologies in agonistic and antagonistic ways [219,220,227,229,231]. Understanding the impact of cross-kingdom interactions on fungal virulence requires a holistic examination of both microbial partners and also the host response. For example, siderophores secreted by *Pseudomonas aeruginosa* that enhance bacterial growth and block neutrophil function also inhibit the germination and growth of *Aspergillus fumigatus* and *Rhizopus* and *Mucor* species, and can even enhance the fungicidal activity of fluconazole against *Candida albicans* [232,233]. Bacteria can also enhance fungal pathogenesis through the establishment of endosymbiotic or endofungal relationships. For example, *Ralstonia pickettii* can reside within *Rhizopus microsporus,* enhancing cell wall-mediated stress resistance, and producing a secondary metabolite that blocks phagocytosis of the fungus [126]. Endofungal bacteria are well established in microbial soil communities [234] , but we only have a limited understanding of their impact on human disease.

Table of content:

In this Review, Brown et al. provide an overview of fungal pathobiology from the pathogen, host and clinical perspectives, focussing specifically on pathogens that can cause invasive life threatening infections.