Menacing Mold: Recent Advances in *Aspergillus* Pathogenesis and Host Defense

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Abstract

The genus *Aspergillus* is ubiquitous in the environment and contains a number of species, primarily *A. fumigatus,* that cause mold-associated disease in humans. Humans inhale several hundred to several thousand *Aspergillus* conidia (i.e., vegetative spores) daily and typically clear these in an asymptomatic manner. In immunocompromised individuals, *Aspergillus* conidia can germinate into tissue-invasive hyphae, disseminate, and cause invasive aspergillosis. In this review, we first discuss novel concepts in host defense against *Aspergillus* infections and emphasize new insights in fungal recognition and signaling, innate immune activation, and fungal killing. Second, the review focuses on novel concepts of *Aspergillus* pathogenesis and highlights emerging knowledge regarding fungal strain heterogeneity, stress responses, and metabolic adaptations on infectious outcomes. Mechanistic insight into the host–pathogen interplay is thus critical to define novel druggable fungal targets and to exploit novel immune-based strategies to improve clinical outcomes associated with aspergillosis in vulnerable patient populations.

Introduction

Fungal pathogens represent a substantial public health risk with more than one million attributable fatalities worldwide annually [1]. The limited number and diversity of contemporary antifungal drugs (i.e., three major drug classes that target two biosynthetic pathways), the emergence of drug resistance in human pathogenic fungi, the lack of vaccines to prevent fungal disease, and the growing number of humans that live in immune-compromised states have all contributed to the emergence of a broad range of invasive mycoses. The latter factor is most critical to understanding fungal diseases attributable to filamentous molds in general, and to the genus *Aspergillus* in particular.

There are more than 200 *Aspergillus* species on Earth, although only a handful are associated with human disease (see Table 1). Like other *Aspergillus* species, *A. fumigatus*, the most common agent of invasive aspergillosis (IA), releases asexual spores called conidia into the air. Humans inhale hundreds to thousands of these infectious propagules on a daily basis. Due to their small size (2–3 μm diameter), *A. fumigatus* conidia can bypass mucociliary clearance and lodge in the lower respiratory tract. In immunocompetent individuals, the coordinated action of the respiratory epithelium, lung-resident macrophages, and recruited neutrophils and monocytes clear conidia efficiently.

The hallmark of IA is the germination of inhaled conidia and the ensuing growth of highly destructive filamentous hyphae that can disseminate hematogenously to remote sites. The term IA does not refer to a specific site of disease, although the respiratory tree is the most common site of infection and the primary focus of this review [2]. Innate immune suppression or injury is a major risk factor associated with the development of IA.

Specifically, profound and prolonged neutropenia and monocytopenia, common side effects of myelotoxic chemotherapy regimens and hematopoietic

Table 1. Common human disease states associated with *Aspergillus*

Disease	Clinical syndromes	Common patient populations and notes
Invasive aspergillosis (tissue-invasive hyphae ± angioinvasion)	Pneumonia and systemic disease; CNS most common site of dissemination	Patients with acute leukemia, lymphoma, myelodysplastic syndrome, aplastic anemia, and allogeneic hematopoietic cell transplant recipients; lung and heart transplant recipients Most common species: *A. fumigatus* (~60%–70%), *A. flavus, A. niger, A. terreus, A. versicolor, A. ustus, A. lentulus, A. nidulans* (only in chronic granulomatous disease patients)
Aspergillus keratitis	Ocular disease caused by hyphal growth in humans with corneal damage	Agricultural workers in resource-limited countries
Chronic pulmonary aspergillosis (hyphae in pre-existing lung cavity)	Pneumonia, typically observed with structural lung disease	Chronic obstructive lung disease, bronchiectasis, sarcoidosis, previously treated tuberculosis
Aspergillus-associated allergic disease	Allergic bronchopulmonary aspergillosis (ABPA), extrinsic allergic alveolitis	ABPA occurs primarily in patients with atopic asthma and cystic fibrosis

cell transplantation, predispose to IA in human cohorts and in high-inoculum mouse models of disease [3]. Chronic granulatomous disease (CGD), caused by a defect in NADPH oxidase, represents a primary immune deficiency with a functional rather than numeric defect in neutrophil function and is also associated with IA susceptibility. Similarly, severe pharmacologic impairment of myeloid cell function, for example, by prolonged corticosteroid exposure, renders patients vulnerable to IA [3]. In most instances, the combination of multiple simultaneous insults to the innate immune system (e.g., myelotoxic chemotherapy, corticosteroids, and small-molecule inhibitors of myeloid function) defines patients at high risk of IA.

In these vulnerable patient groups, failure of the respiratory innate immune system to prevent conidial germination sets the stage for invasive disease [4]. Despite contemporary antifungal drugs, an estimated 200,000 cases of IA occur globally [5], with fungus-attributable mortality rates that exceed 20% in patients with hematologic malignancies and in allogeneic hematopoietic cell transplants [6]. Patients that receive ibrutinib [7] for B-cell malignancies, chronic obstructive pulmonary disease patients, and intensive care unit patients, in particular critically ill patients with influenza, are increasingly recognized as being vulnerable to IA [8,9]. In this review, we summarize recent developments in antifungal immunity and fungal pathogenesis, with an emphasis on studies conducted since 2016. For a review of *Aspergillus*-associated allergenic and toxin-mediated disease states, the reader is referred to the following publications [10–12]. Beyond the common pathogenic species of *A. fumigatus*, *A. flavus*, *A. terreus*, *A. niger*, and *A. nidulans*, the following publication highlights rare and emerging *Aspergillus* species, primarily seen in patients with primary immune deficiency diseases [13].

The Host Response to *Aspergillus*

Novel advances in *Aspergillus* recognition

The *A. fumigatus* cell surface, like that of other fungal pathogens, consists of a multi-layered, polysaccharide-rich cell wall [14]. The conidial cell wall is covered by a proteinaceous, highly hydrophobic layer of rodlets called hydrophobins, which conceals an underlying fungal pigment, melanin. The 1,8-dihydroxynapthalene (DHN) pathway produces the conidial pigment in *A. fumigatus* and *A. terreus*, while the 3,4-dihydroxy phenylalanine pathway operates in *A. niger* and *A. flavus*[15]. These outer layers are shed during conidial germination, exposing a core layer of β-1,3-linked glucan polysaccharides, mannoproteins, and galactomannan, all of which activate pattern recognition receptors of the innate immune system. The inner layer of the polysaccharide-rich cell wall consists of chitin, a polymer of *N*-acetylglucosamine; this layer resides immediately above the plasma membrane [16]. Hyphae lack the hydrophobin and melanin layer and synthesize an external layer of galactosaminogalactan, which is absent from conidia (Fig. 1) [17].

The C-type lectin family of receptors (CLR) has emerged as a critical point of response to fungal pathogens and specifically *A. fumigatus*. Conidial germination is the major trigger for innate immune activation, with a dominant role of Dectin-1/*CLEC7A* signaling in response to *A. fumigatus* β-1,3-glucan exposure [18,19]. The carbohydrate-binding domain of Dectin-1 recognizes particulate β-1,3-glucans, a process that leads to Src kinase-dependent phosphorylation of its intracellular ITAM-like motif [1,20] and to the activation of spleen tyrosine kinase (Syk). Syk activation then promotes the assembly of a trimeric complex of CARD9, BCL10, and MALT1, which is essential for the production of NF-κB-

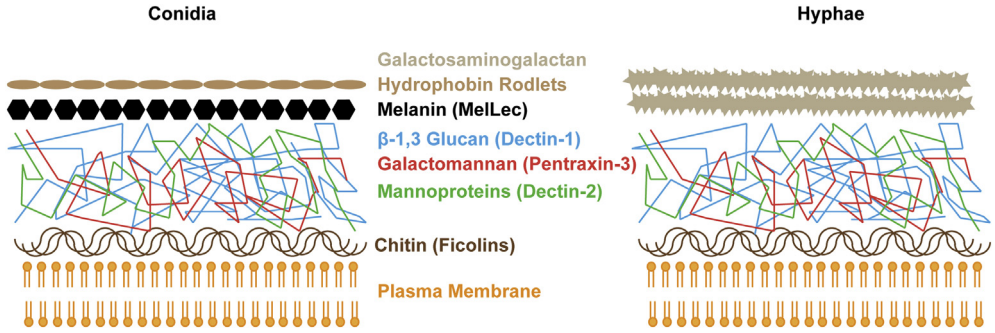

Fig. 1. Schematic of the *A. fumigatus* cell wall. The composition of the *A. fumigatus* cell wall depends on the fungal morphotype. In conidia, a hydrophobin and melanin layer shields the immunogenic core of the cell well, consisting of carbohydrates and glycoproteins. Germlings and hyphae lose the outer conidial layer, leading to exposure of immunoreactive polysaccharides and the eventual synthesis of an outer galactosaminogalactan layer. The indicated receptors trigger innate immune activation in response to exposure of defined polysaccharide and melanin ligands.

dependent cytokines by myeloid cells in Aspergillus-infected mice, including pro-IL1β, TNF, IL-17A, and IL-22 [21–24]. Dectin-1/Syk activation also activates NFAT transcription factors that regulate additional receptors, for example, FcεRI/CD23/*CLEC4J*, implicated in fungal control (see below).

Dectin-1/Syk signaling also leads to the assembly of inflammasomes, multiprotein complexes that include a NOD-like receptor family member (i.e., NLRP3 or AIM2), a common structural component (ASC), and a caspase moiety [25–27] (Fig. 2). In the case of *A. fumigatus,* NLRP3 inflammasomes relevant for host defense consist of a ternary complex of NLRP3, ASC, and caspase-1; the resulting complexes lead to the caspase-1-dependent proteolytic processing of pro-IL-1β into bioactive IL-1β. The non-canonical caspase-8 can be recruited to the CARD9/BCL10/MALT1 complex with ASC, resulting in an alternate IL-1β processing pathway [28]. A recent study suggested that caspase-11 may activate caspase-1 and thus regulate IL-1β release during *A. fumigatus* infection [29]. Consistent with a role for inflammasome activity in anti-*Aspergillus* defense, corticosteroid-treated NLRP3-, AIM2-, or caspase-1 deficient mice are more susceptible to *A. fumigatus* challenge compared to corticosteroid-treated controls [27,29,30]. However, in otherwise immune-competent mice, IL-1β production appears dispensable for host defense against respiratory *A. fumigatus* challenge [31]. Instead, the inflammasome-independent production and release of IL-1α appears critical for host defense in this setting [31].

IL-1α and IL-1β trigger IL-1 receptor/MyD88 signaling in pulmonary epithelial cells to promote the initial -CXC- chemokine-mediated influx of neutrophils into the *A. fumigatus*-infected lung [21]. Sustained neutrophil recruitment relies on CARD9 signaling in hematopoietic cells; this amplifies the IL-1R/MyD88-dependent and CARD9-independent production of neutrophil chemotactic mediators by the lung epithelium. CARD9 function is dispensable for neutrophil-intrinsic fungal killing in mice and humans [21,32,33]. Humans with germline mutations in CARD9 do not develop IA at pulmonary sites, consistent with the idea that CARD9-dependent neutrophil recruitment can be functionally compensated by CARD9-independent mechanisms in the lung. However, outside the lung, a gross absence of neutrophil recruitment has been reported in a case of abdominal aspergillosis, suggesting that neutrophil recruitment depends heavily on CARD9 signaling at extrapulmonary sites [21], similar to findings reported for neutrophil chemotaxis to the *Candida*-infected central nervous system [34].

A. fumigatus triggers Dectin-2/*CLEC4N* activation, although the precise fungal ligand on swollen conidia and germlings that binds to the Dectin-2 carbohydrate recognition domain remains unknown [34]. In other fungal organisms, cell wall mannan structures trigger Dectin-2 activation [35]. Dectin-2 signals via an adaptor protein FcRγ and activates CARD9-dependent signals [34]. In a fungal keratitis model, systemic exposure to *Aspergillus* antigens gives rise to Dectin-2^+ Rorγt$^+$ IL-17RC$^+$ neutrophils in an IL-6- and IL-23-dependent manner; this activated neutrophil subset produces the effector cytokine IL-17A, leading to autocrine activation and heightened fungicidal responses [36]. Similarly, *Aspergillus* stimulation leads to increased macrophage Dectin-2 expression in a pulmonary infection model [37]. However, the precise contribution of Dectin-2 to *in vivo* host defense against *A. fumigatus* remains poorly defined and may, at least part, explain the more severe human and murine phenotype of CARD9 deficiency compared to Dectin-1 deficiency [32]. The potential contributions of the FcRγ-coupled CLRs Mincle/*CLEC4E* and Dectin-3/MCL/*CLECSF8*/*CLEC4D* to *in vivo* host defense against *Aspergillus* remain undefined.

CD23/FcεRI/*CLEC4J*, the low-affinity receptor for IgE, is a FcRγ-coupled C-type lectin that binds to fungal β-glucan and α-mannan moieties and regulates NF-κB-dependent macrophage cytokine and nitric oxide production. Notably, CD23 deficiency

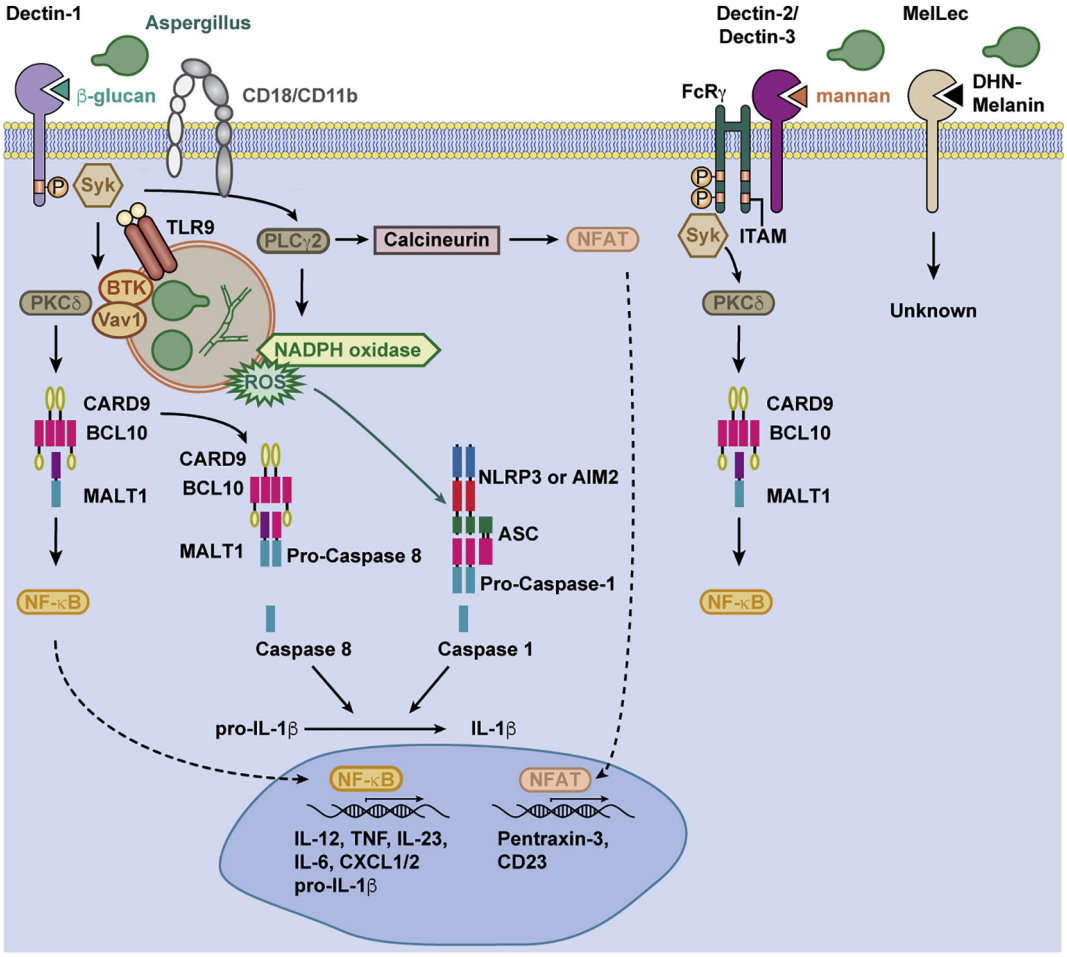

Fig. 2. *Aspergillus* ligands activate C-type lectin receptor signaling in myeloid cells. *A. fumigatus* β-glucans activate Dectin-1 and CR3 signaling, resulting in Syk phosphorylation. *A. fumigatus* can activate the FcRy-coupled receptors Dectin-2 and Dectin-3 via mannan moieties, and MelLec via DHN-melanin exposure. Dectin-1/Syk and Dectin-2/Syk activation is transduced via PKCδ and CARD9 for assembly of the ternary CARD9/BCL10/MALT1 complex, NF-κB activation, and the transcription of NFκB-dependent genes, including pro-IL-1β and other cytokines. The assembly of NLRP3 and AIM2 inflammasomes results in proteolytic activation of caspase-1 and the production of bioactive IL-1β. Alternatively, ASC and caspase-8 recruitment to the CAD9/BCL10/MALT1 complex gives rise to active caspase-8 and bioactive IL-1β. CR3/Syk-dependent PLCγ2 activation is linked to NADPH oxidase assembly, the production of reactive oxygen species, and calcineurin-dependent NFAT activation. NFAT-dependent genes include pentraxin-3 and CD23. Dectin-1 can interact with VAV1 and BTK to facilitate macrophage fungal phagocytosis. BTK may also promote calcineurin activation and the production of NFAT-regulated cytokines. The model depicts fungal killing in the phagosome.

results in impaired survival in an intravenous *A. fumigatus* murine infection model, although it is likely dispensable for host survival and fungal clearance during pulmonary infection [38].

Melanin-sensing C-type lectin receptor (MelLec/*CLEC1A*) is expressed on endothelial, but not myeloid, cells in mice and on myeloid cells in humans. MelLec recognizes *A. fumigatus* conidial DHN-melanin, and its downstream signaling cascade remains to be characterized. In the absence of MelLec, otherwise immunocompetent mice are susceptible to systemic, but not pulmonary, infection [39]. Beyond MelLec, surfactant protein D (SP-D), a soluble C-type lectin receptor, may recognize melanin. Opsonization of conidia by SP-D stimulates phagocytosis and inflammation *ex vivo*. Moreover, *in vivo*, SP-D deficient mice have impaired cytokine responses, specifically in TNF, IL-6, IL-8, IL-1α, IL-1β, and TGF-β production, following *A. fumigatus* challenge. Collectively, these findings emphasize the role of melanin recognition in host defense against *A. fumigatus*[40].

Complement receptor 3 (CR3), a myeloid cell integrin receptor consisting of CD11b and CD18 subunits, binds β-glucan moieties in addition to its well-defined role in complement recognition [41]. *In vivo*, CD18 knockout mice, a model of human leukocyte adhesion deficiency, fail to recruit neutrophils to infected tissues in an *Aspergillus* keratitis

model and develop more severe disease compared to CD18-sufficient controls [42]. In a pulmonary infection model, CD18-dependent neutrophil recruitment is not grossly impaired and CD18-deficient mice do not exhibit an overt susceptibility defect, highlighting tissue-specific requirements for CD18 in neutrophil trafficking and fungal killing during *A. fumigatus* infection [32]. This is not specific to *A. fumigatus* lung infections, as pulmonary neutrophil recruitment in response to *Escherichia coli* pneumonia also does not require CD18 [43]. CR3 has also been implicated in the *A. fumigatus*-triggered neutrophil respiratory burst and hyphal killing [42,44], as discussed below.

The soluble pattern recognition receptors Pentraxin-3 and M-ficolin recognize newly exposed carbohydrate layers during conidial germination. Pentraxin-3 binds to galactomannan, enhances human neutrophil conidial engulfment [45], and is a component of neutrophil extracellular traps (NETs) [46]. Ficolin-deficient, but otherwise immunocompetent mice display normal neutrophil recruitment as measured by lung myeloperoxidase levels, as well as normal survival, yet exhibit delayed fungal clearance and reduced IL-1β, IL-6, and TNF responses in infected lung tissues [47]. The C-type lectin receptor DC-SIGN, expressed primarily on human alveolar macrophages, recognizes galactomannan as well and likely contributes to fungal cell phagocytosis. Soluble receptors, including the ficolin family of receptors, also recognize the inner chitin layer once exposed [48]. Lung epithelial cells express the membrane bound protein fibrinogen C domain-containing protein 1 (FIBCD1). FIBCD1 recognizes chitin on the fungal cell wall and can suppress the production of IL-8, mucin, and other pro-inflammatory mediators [49]. The cell wall component galactosaminogalactan also maintains immunomodulatory functions by concealing immunogenic cell surface components from their receptors, such as shielding β-glucans from Dectin-1 [50].

Fucosylated host glycoproteins have recently emerged as important components in *Aspergillus* recognition, by virtue of their binding to the fungal lectin FleA. FleA acts as a ligand for fucosylated glycoproteins in purified lung mucin and regulates fucose-mediated conidial binding and uptake by macrophages. In the absence of FleA, macrophages are defective at internalizing conidia *in vitro* and mice infected with FleA-deficient conidia develop more severe lung infection than mice infected with FleA-sufficient conidia, establishing fucosylated structures as critical for *Aspergillus* recognition and clearance [51].

Importantly, human polymorphisms in the *CLEC7A* (~20% IA incidence *versus* ~15% in the control group at 24 months post-transplant), *PTX3* (~20% IA incidence *versus* ~15% in the control group at 24 months post-transplant) [52], and *CLEC1A* (39% IA incidence *versus* 17% in the control group at 24 months post-transplant) [39] increase the risk of IA in allogeneic hematopoietic cell transplant recipients, although these genetic variants are not associated with the spontaneous development of IA in otherwise immune-competent individuals. Humans with genetic defects in Toll-like receptor signaling similarly do not develop fungal disease in the absence of other risk factors [53]. In murine models, deficiencies of TLR2 and TLR4 have been associated with murine susceptibility to *A. fumigatus* challenge in the context of pharmacologic immune suppression [54,55]. In humans, a *TLR4* S4 haplotype influences susceptibility to IA in allogeneic HCT patients (22% IA incidence with S4 haplotype *versus* ~1%–10% in the control group, depending on the presence of CMV disease), but it remains unclear whether TLR4 binds and responds to *A. fumigatus* directly or, more likely, that this *TLR4* haplotype is linked to a general alteration in innate immune reactivity to inflammatory stimuli [56].

Bruton's tyrosine kinase, ibrutinib, and the risk of IA

Bruton's tyrosine kinase (BTK) transduces signals from the B-cell receptor (BCR), and this kinase has been targeted successfully with ibrutinib to inhibit tonic BCR signaling required for the maintenance of malignant B cells. Beyond this established role in BCR signaling, BTK acts downstream of Dectin-1- and TLR-dependent signaling in myeloid cells during interactions with fungi (Fig. 2). In *A. fumigatus*-infected macrophages, endosomal TLR9 activates calcineurin signaling in a MyD88-independent and BTK-dependent manner to regulate NFAT-dependent TNF production [57]. Since Pentraxin-3 is an NFAT-dependent gene, this finding may explain why impaired calcineurin signaling in DCs predisposes to IA in a murine allogeneic hematopoietic cell transplant model of graft *versus* host disease [58]. Beyond its function in macrophage cytokine production, BTK also plays a role in Dectin-1-dependent macrophage *C. albicans* phagocytosis [59].

Recently, several reports observed that lymphoma patients treated with ibrutinib, a BTK inhibitor, exhibit susceptibility to IA, particularly in the central nervous system, even in the general absence of well-defined concurrent clinical risk factors such as neutropenia or corticosteroid exposure [7,60,61]. Although the studies examined heterogenous patient groups, ibrutinib treatment resulted in an IA incidence rate of 0.5%–39%, depending on the presence of concurrent chemotherapy, with most reported incidence rates below 5% [62]. These clinical findings support the concept that BTK signaling and inhibition by ibrutinib cause a defect in fungal immune surveillance. Consistent with this model, *BTK*-deficient mice are more vulnerable to *A. fumigatus* challenge compared to controls [7]. In addition, ibrutinib impairs NFκB/NFAT signaling during macrophage *A. fumigatus*

phagocytosis [63]. While it remains unclear whether ibrutinib has BTK-independent effects on fungal immune surveillance, these data support the idea that BTK signaling in myeloid cells contributes to anti-*Aspergillus* host defense [59].

Innate immune cells in anti-*Aspergillus* immunity

As outlined above, neutrophils and monocytes are essential cellular components of anti-*Aspergillus* immunity in humans and mice [64–67], highlighting the essential role of the innate rather than adaptive immune system in mediating sterilizing immunity. Adaptive immune responses to *A. fumigatus* are reviewed elsewhere [68]. In mouse models, macrophages kill conidia and contribute to fungal clearance as well [32,69], although pulmonary macrophage ablation experiments reveal that loss of their antifungal activity can be functionally compensated [64]. Monocytes and monocyte-derived dendritic (MoDCs) cells both kill conidia and, via intercellular cross-talk, enhance the fungicidal properties of recruited neutrophils [65]. Type I and III interferon (IFN) signaling has emerged as central to this process. Lung-infiltrating $CCR2^+$ inflammatory monocytes regulate the local and rapid production of IFN-α, which in turn stimulates IFN-λ production. Neutrophils express type I and type III IFN receptors that activate STAT1 signaling and promote the fungicidal respiratory burst [70]. Exogenous stimulation of type I IFN production leads to improved survival of CGD mice upon *A. fumigatus* challenge [71], suggesting that type I IFN may be beneficial even in the context of an impaired neutrophil respiratory burst.

Granulocyte–macrophage colony-stimulating factor (GM-CSF) plays an important role in intercellular crosstalk and neutrophil activation as well. Neutrophils that cannot sense GM-CSF exhibit an impaired oxidative burst, and therapy with recombinant GM-CSF accelerates *Aspergillus* clearance in an NADPH oxidase-dependent manner [72]. Another hematopoietic growth factor, macrophage colony-stimulating factor, accelerates myeloid cell differentiation after allo-HCT in mice and improves survival if a mouse is infected 1 week post-transplant [73].

A recent paper indicated that a minor neutrophil subset expresses the integrin CD11c and the major histocompatibility complex class II receptor during respiratory fungal infection. Termed neutrophil-DC hybrids, this rare population is more efficient at fungal cell uptake and killing compared to canonical $CD11c^-$ major histocompatibility complex class II^- neutrophils [74]. Because tools to manipulate neutrophil-DC hybrids *in vivo* have not been established, it remains challenging to quantify their contributions to fungal clearance.

Plasmacytoid dendritic cells (pDCs) contribute to innate defense as well, since antibody-mediated depletion renders mice susceptible to *A. fumigatus* challenge [75]. pDCs express Dectin-2 and are activated in response to *Aspergillus* antigens, although the pDC transcriptional response appears distinct to that elicited by microbial nucleic acids, typically observed in viral infections [76]. pDCs have been proposed to form rare extracellular traps following *A. fumigatus* exposure. A model that integrates pDC trafficking and function in the immune response to *A. fumigatus* has not yet been established [76].

In immune-competent mice, T cells, B cells, innate lymphoid cells, and natural killer (NK) cells do not play an essential role in host defense because RAG2- and IL-2 receptor common γ chain-deficient mice do not exhibit enhanced sensitivity to respiratory challenge [65]. However, NK cells may play beneficial roles in fungal clearance in the setting of injury to the myeloid cell compartment, as shown in a neutropenic model of IA [77]. NK cells express CD56, a 140-kDa subunit of the human neural-cell adhesion molecule. CD56 appears to interact directly with *A. fumigatus*, leading to a reduction in surface expression. Blocking CD56 impairs NK cell activation and cytokine secretion, consistent with the idea that CD56 is a fungal pattern recognition receptor for NK cells [78]. *In vitro* stimulation of NK cells by *A. fumigatus* germlings leads to an increase in inflammatory cytokine production and granule polarization, and there is an increase in fungal DNA release, which is indicative of fungal damage. However, granule release is impaired upon contact with germlings. In agreement with this observation, co-incubation of *A. fumigatus* with NK cells and leukemic cells results in an impaired NK response to the leukemic cells. This finding suggests that fungi may dampen NK cell cytolytic activity and induce an exhaustion-like phenotype [79]. DCs exposed to *A. fumigatus* antigens have the capacity to stimulate NK cells in a contact-independent manner [80].

In vitro experiments support a potential regulatory role for platelets in anti-*Aspergillus* host defense [81]. The addition of platelet-rich plasma to macrophage-*Aspergillus* co-cultures significantly increases fungal phagocytosis by macrophages. In addition, platelet-rich plasma reduces fungal metabolic activity in both DC-*Aspergillus* and macrophage-*Aspergillus* co-culture experiments compared to control conditions [82]. In an *in vitro* model of platelet coagulation, co-culture of *A. fumigatus* conidia and germlings led to the formation of conidia-platelet aggregates surrounded by neutrophils [83]. Although the *in vivo* role of platelets remains unclear, thrombocytopenia has been identified as a risk factor for IA outcomes in liver transplant patients [84] and in neutropenic patients undergoing induction chemotherapy for hematologic malignancies [85,86].

Trained immunity is a memory-like phenotype observed in innate immune cells upon serial exposure

to fungal ligands, exemplified by β-glucans and resistance to *C. albicans* infection [87]. Trained monocytes exhibit metabolic adaptations, including high glucose consumption, lactate production, and an increased NAD^+ to NADH ratio, which is indicative of a change in metabolism toward glycolysis. This supports increased pro-inflammatory responses upon subsequent encounter with fungal cell wall antigens [88]. *In vivo*, an initial exposure to live or killed *Aspergillus* antigens protects against secondary challenge in mice that lack adaptive immune cells. This protective phenotype depends on IL-17 signaling and is associated with enhanced CXCR2 signaling-dependent recruitment of neutrophils to the site of infection, as well as heightened microbicidal activity [89].

The importance of the microbiota in anti-*Aspergillus* defense remains poorly understood. However, a recent study established that the composition of the intestinal microbiome appears to influence the formation of $CD4^+$ T helper 17 cells in the lung following pulmonary *Aspergillus* infection [90]. Much more work will be necessary to understand how either local (pulmonary) or distant (extrapulmonary) microbial communities influence host susceptibility to inhaled *Aspergillus* spores and to *Aspergillus*-associated allergenic disease states.

Novel insights into fungal killing

Myeloid cells kill *Aspergillus* by oxidative and non-oxidative mechanisms and employ different strategies to kill conidia and hyphae. Following phagocytic uptake, conidial killing typically occurs within phagolysosomes. Murine neutrophils employ NADPH oxidase to kill conidia in a cell-intrinsic manner. However, NADPH oxidase-deficient neutrophils have a partial conidial killing defect, consistent with of alternative, non-oxidative mechanisms [32]. In contrast, hyphal killing occurs predominately in the extracellular space and may coincide with NET formation [91], a process that extrudes genomic DNA, citrullinated histones, antimicrobial peptides, pentraxin-3, and calprotectin around *Aspergillus* hyphae. Dectin-1 and CR3 participate in this bifurcated response to fungal cell size by regulating the process of NET formation [44,92]. Although neutrophil contact with *A. fumigatus* hyphae induces NET formation, available experimental evidence does not support a direct fungicidal activity of these structures [44,93,94].

A recent study demonstrated that neutrophil-derived reactive oxygen species induce an apoptosis-like regulated cell death (A-RCD) in leukocyte-engulfed conidia [95]. In other words, leukocytes trigger a gene-dependent cell death program in *A. fumigatus* via the action of NADPH oxidase, consistent with the susceptibility of CGD patients to IA. This process is inhibited by the action of *AfBir1*, a negative fungal regulator of A-RCD. *AfBir1* is a homolog of the human *SURVIVIN* gene, a member of the inhibitor-of-apoptosis (IAP) protein family. IAP proteins in humans and fungi encode conserved BIR domains that, in the case of the human protein XIAP, can suppress mammalian apoptosis by blocking caspase activation [96,97]. Consistent with a role as a negative regulator in fungal RCD, *AfBir1* overexpression diminishes leukocyte-induced conidial A-RCD and increases virulence in a murine model of IA. In contrast, presumed pharmacologic inhibition of *AfBir1* diminishes conidial viability in neutrophils and leads to more rapid fungal clearance *in vivo*. A major gap in knowledge relates to the identity of fungal effector genes that mediate the A-RCD response [98–100].

Like their murine counterparts, human neutrophils employ different mechanisms to kill conidia and hyphae. As stated above, CR3 recognizes conidia and can regulate neutrophil killing by phosphoinositide 3-kinase (PI3K)-dependent mechanisms at high effector to target cell ratios [93]. In this study, the authors suggest that CR3- and PI3K-dependent conidial killing does not require the oxidative burst. Human neutrophils isolated from CGD patients can employ iron sequestration as a form of nutritional immunity (see below) to inhibit fungal growth [101]. In contrast to conidial killing, the killing of *A. fumigatus* germlings and hyphae requires antibody opsonization and neutrophil signaling through Fcγ receptor, SYK, PI3K, and PKC to generate fungicidal ROS through NADPH oxidase [93]. Differences in experimental observations are likely reconciled by redundancies in oxidative and non-oxidative conidial killing mechanisms, potential differences in the dependency of human *versus* murine neutrophils on specific effectors, and *in vivoversusex vivo* experimental conditions.

In macrophages and neutrophils, NADPH oxidase activity promotes non-canonical autophagy known as LC3-associated phagocytosis (LAP) [102–104]. With regard to *A. fumigatus*, the presence of conidial melanin inhibits LAP. Whether LAP represents a critical defense mechanism *in vivo* remains poorly understood; hematopoietic *Atg5*-deficient mice have defects in macrophage-dependent conidial killing but are not vulnerable to *A. fumigatus* challenge in the absence of exogenous pharmacologic immune suppression [105].

Aspergillus Pathogenesis

Animal models and *Aspergillus* pathogenesis

Many animal studies on IA have examined disease outcomes in immune-competent mice to avoid pleiotropic effects of pharmacologic immune suppression or in chemotherapy- or corticosteroid-treated mice to mimic classic human risk factors, related to quantitative or qualitative myeloid cell injury associated with

disease development [106]. A drawback of studying immune-competent mice is their resistance to disease development [107], and the typical range of infectious inocula typically varies from 10^7 to 10^8 conidia [108]. In contrast, immunocompromised mice typically receive a lower inoculum that usually ranges from 10^4 to 10^7 conidia [109].

Neutropenic mice develop extensive and rapid hyphal growth that is angioinvasive and results in tissue necrosis [109]. Consistent with this numeric defect, adoptive transfer of $CD11b^+$ phagocytes ameliorated IA outcomes in the neutropenic cyclophosphamide model. Corticosteroid-treated animals develop less invasive hyphal growth than cyclophosphamide-treated mice, in part due to massive infiltration of dysfunctional neutrophils, a process that leads to pronounced tissue hypoxia (see below). Accordingly, adoptive transfer of $CD11b^+$ leukocytes does not improve infectious outcomes in this model [110]. CGD mice lack neutrophil NADPH oxidase activity and develop immune responses to *A. fumigatus* challenge that more closely resemble those observed in corticosteroid-treated mice than in neutropenic mice, because the extensive hyphal growth and angioinvasion seen in neutropenic mice is generally blunted in corticosteroid-treated or CGD mice. In addition, *A. fumigatus* produces gliotoxin, a fungal secondary metabolite that can induce regulated cell death in host cells, more readily in corticosteroid-treated and CGD mice than in neutropenic mice [109]. Dysregulated immune function is observed in mice with defects in the cystic fibrosis transmembrane regulator. Although CF patients do not develop IA, their airways are commonly colonized with *Aspergillus* and allergic bronchopulmonary aspergillosis is common in this patient group [111].

In mice with qualitative immune defects, researchers have examined infectious outcomes in the context of dampening fungus-induced inflammatory responses to minimize tissue damage. For example, although epithelial IL-1 receptor signaling is essential for neutrophil recruitment and fungal clearance in immune-competent mice [31], dampening IL-1 signaling with an IL-1 receptor antagonist (i.e., anakinra) slightly extends survival in corticosteroid-treated mice [112]. This result highlights a paradox: at early stages of infection, IL-1 receptor signaling is essential for orchestrating the initial neutrophil recruitment to the site of fungal infection. Although anakinra treatment is not typically associated with susceptibility to invasive fungal infections, *Aspergillus* pneumonia has been reported in a patient treated with this agent for Still's disease [113]. However, the results reported in Ref. [112] support the idea that unchecked IL-1 signaling may exacerbate infectious outcomes at later stages of infection in the context of functionally defective neutrophils.

The idea that excessive IL-1 signaling may be detrimental to the host has been extended to a murine CGD model, in which the pleiotropic cytokine IFN-γ, dispensable for fungal clearance in otherwise immune-competent mice [70], promotes LC3-associated autophagy (discussed above) and activates the death-associated protein kinase 1 (DAPK1) pathway. DAPK1 inhibits NLRP3 inflammasome activation by proteosomal degradation, thereby attenuating downstream IL-1β production. In a murine CGD model, DAPK1 protein levels are low and LAP is attenuated due to its dependency on NADPH oxidase activity. Administration of exogenous IFN-γ restored DAPK1 activity, reduced IL-1β production, and increased fungal clearance in this model. In allo-HCT patients, *DAPK1* promoter single nucleotide polymorphisms that lead to diminished protein expression were associated with an increased IA risk (42.1% IA incidence in patients with *DAPK1* polymorphism *versus* 17.4% IA incidence in control patients) [114]. These results support the notion that unrestrained IL-1 signaling may be harmful in specific host contexts. Along these lines, anakinra administration improves outcomes in CGD mice by inhibiting excess neutrophil recruitment and, paradoxically, fungal growth [115]. How to integrate these findings into treatment strategies remains a complex challenge, given the context-specific beneficial and detrimental effects of IL-1 signaling in IA outcomes.

Strain variability and virulence

In recent years, strain selection has emerged as an important variable in *Aspergillus* pathogenesis research. Classically, *Aspergillus* pathogenesis has been analyzed and understood in terms of defined pharmacologic, genetic, or numeric deficits in host immune function. Beyond host immune injury, the inoculum represents an important additional variable to determine fungal clearance, since a mathematical model predicted that low-dose infections, the most common occurrence in human life, would lead to fungal persistence compared to high dose infections [116].

Recent studies that compared the virulence and pathogenesis of different clinical isolates have begun to expand this view. Large-scale genome comparisons of *A. fumigatus* isolates did not identify specific virulence factors shared with other human fungal pathogens. However, the identification and functional characterization of strain-specific metabolic and adaptive traits critical for growth, survival, and competition within its natural habitat (i.e., decaying organic matter) form the basis for *Aspergillus* pathogenesis in human hosts [117]. More recently, the concept of strain-specific virulence traits has emerged within this framework and represents an active area of investigation.

In animal models of IA, two major clinical isolates are commonly utilized: AF293 (the original reference

Table 2. Comparison of *A. fumigatus* CEA10 and AF293 strains

Strain	*CEA10*	*AF293*
Virulence	High	Low
Germination	Fast	Slow
Elimination in the lung	More rapid	Less rapid
Lung inflammation	Higher levels	Lower levels
Hypoxia fitness	High	Low, can be increased by serial passaging in hypoxia

genome strain [118] that was isolated from patient with rheumatoid arthritis, see NCPF 7367) and CEA10 (a strain isolated from a patient with acute leukemia, see FGSC A1163) (Table 2). AF293 is the less virulent and CEA10 the more virulent of these strains within the murine lung environment [119]. However, following experimental challenge in the mouse lung, CEA10 is cleared more rapidly than AF293, consistent with the concept that different strains induce qualitatively and quantitatively distinct host responses [120], leading to differences in clearance kinetics or in disease development in immune-competent or susceptible hosts, respectively.

A consistent phenotype of the more virulent CEA10 strain is the more rapid conidial germination and hyphal growth *in vitro* and *in vivo*, observed in zebrafish and in mice [121], compared to AF293. The more rapid reduction in fungal burden seen with the CEA10 strain [120] is accompanied by a broader induction of inflammatory mediators *in vitro* and *in vivo*, in particular mediators associated with lung damage (i.e., IL-1α) and neutrophil chemotaxis (i.e., TNF, CXCL1, CXCL2, leukotriene B4) [122,123]. *Alox5* null mice are unable to metabolize arachidonic acid to leukotrienes and are thus significantly more susceptible to CEA10 than AF293 challenge, highlighting the induction of and requirement for specific chemotactic pathways in a strain-specific manner [123].

Although AF293 can induce macrophage and DC IL-1β release, this strain consistently induces higher levels of the regulatory cytokine IL-10 compared to CEA10 [122] and does not require IL-1α- or leukotriene B4 (LTB4)-dependent neutrophil recruitment for fungal clearance [121]. This finding may also reflect strain-specific induction of lung hypoxia at sites of fungal tissue invasion, since tissue hypoxia drives expression of the transcription factor HIF-1α. In turn HIF-1α controls LTB4 synthesis [123] by regulating the expression of 5-lipooxygenase activating protein, a key enzyme in the leukotriene biosynthesis pathway [124]. Thus, the strain-specific differential induction and quality of inflammatory responses demonstrates an inverse relationship between inflammation-induced host damage and the kinetics of fungal clearance.

The fungal hypoxia adaptation response will be discussed in more detail below, and host hypoxia signaling is critical for murine survival in CEA10 but not AF293 infections [123]. The idea that strain-specific hypoxia fitness impacts virulence is underscored by the differential outcomes in a corticosteroid model of IA, which results in low-oxygen lesions in the lung. In this model, CEA10-infected mice succumb rapidly and significantly more than AF293. In agreement with this model, an experimentally evolved AF293 strain, created by serially passaging an isolate in hypoxia conditions, developed increased hypoxia fitness and displayed much higher virulence than the parental AF293 strain and similar to the CEA10 strain in the corticosteroid model [125]. The genetic basis that underlies this phenotypic evolution remains undefined.

Fungal metabolism and *Aspergillus* virulence

The ability of *A. fumigatus* to scavenge and utilize essential nutrients in a depleted environment impacts the outcome of infection (Fig. 3). The lung airway surface liquid contains low levels of glucose, almost 10-fold lower than in the serum. The levels of ammonium, a preferred nitrogen source for fungi, and free amino acids are very low in the lung environment, unless expanded microbial communities and dysregulated tissue environments are present, for example, in cystic fibrosis patients [126,127]. The glycoproteins of the mucus layer may be good sources of some these macronutrients, in particular because *A. fumigatus*, like most filamentous molds, produces a large number of extracellular proteases to degrade polypeptides [126]. Micronutrients such as metals also are required for growth of fungi [128] and the lung microenvironment contains very low levels of magnesium, calcium, iron, zinc and copper, compared to the bloodstream [129]. The regulation of micronutrient availability at portals of fungal invasion represents a form of nutritional immunity, a process by which the host may restrict the availability of necessary nutrients or flood the pathogen with those that are toxic in excess [130].

Iron is a critical nutrient for *Aspergillus* growth, and mutants with defective iron assimilation pathways are hypovirulent in immune compromised mice [131]. Lung transplant recipients are vulnerable to IA, particularly at anastomotic sites, consistent with the idea that presence of lung microhemorrhages may increase iron availability. In an experimental murine model of lung transplantation, the presence and extent of allograft-associated microhemorrhage, and the topical iron application in syngeneic lung grafts increased susceptibility to infection. When an iron-intolerant *A. fumigatus* strain was used, the enhanced susceptibility to IA disappeared [132].

Given the limited glucose sources in the lung, carbon assimilation is critical to *Aspergillus* growth in this environment. G-protein coupled receptors and hexose transporters have been reported to be involved in glucose sensing and uptake in yeast, and functional homologs exist in *A. fumigatus* [126]. The G-protein

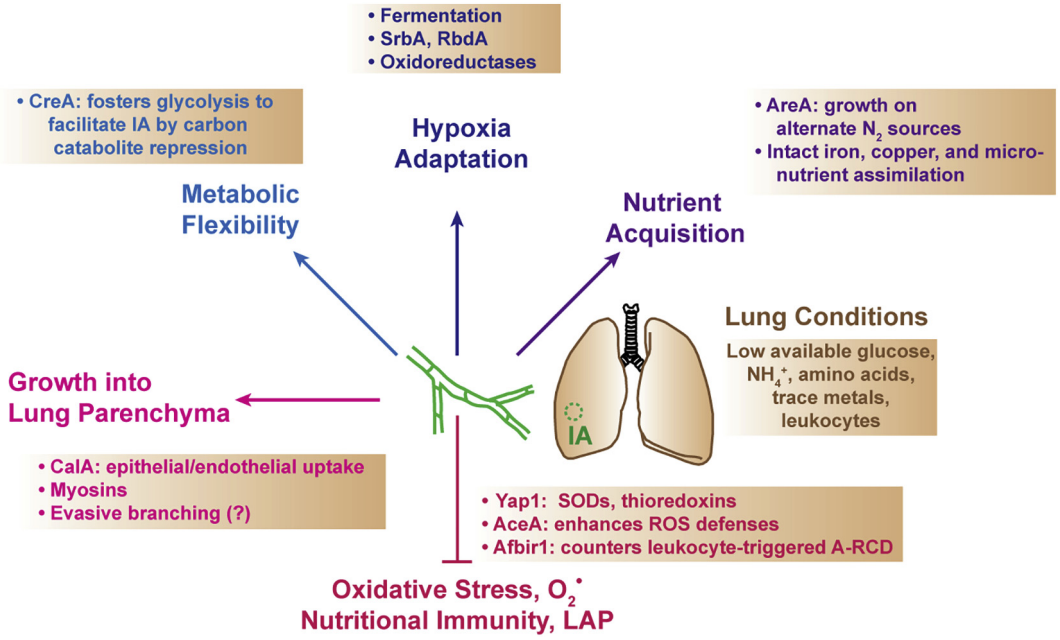

Fig. 3. *Aspergillus* adaptations and gene programs that promote invasive pulmonary aspergillosis. *Aspergillus* can establish IA under specific host conditions by overcoming scarce nutrient availability, exhibiting metabolic flexibility and adaptation of tissue hypoxia, invading the lung parenchyma, and neutralizing oxidative and non-oxidative host defense mechanisms. The panels indicate a selected set of fungal genes and fungal properties that are essential to promote invasive disease and to counter host defense mechanisms. SODs, superoxide dismutases; ROS, reactive oxygen species; A-RCD, apoptosis-like regulated cell death; LAP, LC3-associated phagocytosis.

coupled receptor GprK was recently found to be necessary for *A. fumigatus* proliferation on medium in which pentose was the only carbon source. Loss of this gene impairs oxidative stress tolerance and leads to lower virulence in a *Galleria* infection model, suggesting that carbon accumulation is important for adapting to an infection microenvironment [133].

Carbon catabolite repression refers to the process of switching from non-preferred to preferred carbon sources. The transcriptional repressor CreA mediates a carbon catabolite repression network that is dispensable for early infection establishment. However, CreA is essential for IA progression in a corticosteroid model. Early in the course of IA, infected airways contain adequate levels of oxygen and non-glucose carbon sources for conidia to germinate and form hyphae. At this early stage of infection, fungal carbon catabolite repression is unnecessary for hyphal growth. Following the induction of hypoxia in areas of fungal growth, a change in the availability of carbon sources requires that hyphae switch to glycolysis to support further elongation [134]. Thus, the induction of carbon catabolite repression acts as a disease progression factor and highlights the role of *A. fumigatus* metabolic flexibility for virulence in the lung environment.

Ammonium and glutamine represent preferred sources of nitrogen that *A. fumigatus* can assimilate. The GATA-type transcription factor AreA is required for growth on non-preferred nitrogen sources, and its activity contributes to invasive disease [135]. The *A. fumigatus* genome contains predicted ammonium and amino acid permeases but their roles in disease development and progression have not been characterized [126]. In contrast, amino acid biosynthesis has emerged as an additional metabolic contributor to virulence and pathogenesis. Auxotrophic *A. fumigatus* strains deficient in aromatic amino acid biosynthesis have attenuated growth *in vitro* and greater susceptibility to various forms of stress. This coincides with reduced virulence in both systemic and pulmonary infection of neutropenic mice [136]. The biosynthesis of cysteine, methionine, and histidine is necessary for virulence as well, since auxotrophic strain showed reduced virulence in immune compromised models of IA [137,138]. Beyond carbon and amino acid biosynthetic pathways, loss of vitamin biosynthesis (i.e., pantothenic acid or riboflavin) results in impaired virulence in neutropenic models of immune suppression by both pulmonary and systemic infection [139].

The fungal stress response and virulence

A. fumigatus faces hypoxia and oxidative stress in the lung. Recent work demonstrates that heterogeneity in hypoxia fitness among different *A. fumigatus* isolates correlates with virulence [125]. To adapt to tissue hypoxia, *A. fumigatus* can switch to fermentation through the action of a fungal alcohol dehydrogenase. Although loss of fungal ethanol fermentation does not

affect murine survival, the AlcC null mutant strains grows more slowly in the lung tissue environment than the complemented control strain [140]. To grow in a hypoxic environment, *A. fumigatus* requires expression of the sterol regulatory element binding protein SrbA, a transcription factor that requires proteolytic activation by putative rhomboid homolog (Rbd) [141]. Loss of either gene sensitizes the fungus to hypoxic and to non-hypoxic stressors, including azole drugs, cell wall stress, and low iron conditions. RbdA-deficient strains exhibit abnormal hyphal tip branching and have attenuated virulence in a corticosteroid model of IA [142]. The oxidoreductase HorA, located in mitochondria, is important for electron transport chain function and cellular respiration, and is highly upregulated under hypoxic conditions. The null mutant exhibited an impaired stress response to hypoxia and reductive stress and was hypovirulent in both corticosteroid and cyclophosphamide models of IA [143].

Given the central role of NADPH oxidase in host defense against *Aspergillus*[144], genetic pathways that counter oxidative stress are important for virulence in immune compromised models of IA. Yap1, a ROS-sensing transcription factor, controls multiple aspects of the *A. fumigatus* responses to oxidative stress. Upon exposure to reactive oxygen species, Yap1 regulates the production of superoxide dismutases (that convert superoxide to hydrogen peroxide) and catalases (that convert hydrogen peroxide to water). *In vitro* hyphal growth in the presence of human neutrophils requires the expression of Yap1 and superoxide dismutase; however, catalases are not required [42].

Yap1 also regulates thioredoxin antioxidant pathway that includes Prx1 and Aspf3, both presumed thioredoxin peroxidases or peroxiredoxins. These function by reducing hydrogen peroxide to water. Blocking thioredoxins pharmacologically with the anticancer drug PX-12 sensitizes hyphae to ROS and to neutrophil killing [42]. Loss of Aspf3 loss impairs growth on H_2O_2 and superoxide, and its protein structure implies a direct reductive action. Impaired growth in the null mutant is concomitant with reduced virulence in a corticosteroid model of IA [145]. The actin polymerizing factor cofilin may also contribute the anti-oxidation stress response. Cofilin overexpression results in increased fungal resistance to oxidative stress by regulating the transcription of oxidative stress response genes, including *yap1*, *catA*, *dprA*, *dprB*, and *skn7*[146].

Fbx15, a transcriptional repressor and member of the E3 ubiquitin ligase complex, is transcriptionally induced by oxidative stress and required for growth on H_2O_2 and virulence in an immunosuppressed mouse model of IA. This result is likely due to two factors: first, the interaction of Fbx15 with the ubiquitin SCF E3 ligase machinery and the interaction with the transcriptional repressor SsnF. Under normal circumstances, Fbx15 is phosphorylated and interacts with the E3 complex predominantly in the cytoplasm but also in the nucleus. Phosphorylated Fbx15 assists in SsnF nuclear transport where SsnF can interact with corepressors that target oxidative stress response and secondary metabolite genes. Upon oxidative stress, Fbx15 is dephosphorylated, causes the E3 complex to become less active, and concurrently leads to less nuclear SsnF, either through the nuclear export machinery or through a change in stability. This leads to a derepression of many genes, including those responsible for gliotoxin production, and other putative stress response genes [147].

Translational regulation is also important for *A. fumigatus* oxidative stress resistance. The cytoplasmic messenger ribonucleoprotein granule Afpab1 is required for growth on H_2O_2, and loss of this protein leads to increased susceptibility to phagocyte killing that is likely due to a presumed inability to detoxify ROS *in vitro*. Loss of Afpab1 results in impaired virulence in a corticosteroid and cyclophosphamide model of IA [148].

A. fumigatus has evolved strategies to counter host nutritional immunity that limits the availability of essential micronutrients, such as iron, or harness their toxic properties, for example, copper. Loss of the copper-binding transcription factor AceA reduces *A. fumigatus* resistance to copper and ROS *in vitro* and increases susceptibility to macrophage-mediated killing. Although copper can function in an antioxidant manner by regulating superoxide dismutase activity, it can also generate hydroxyl radicals from H_2O_2 through Fenton Chemistry. In the absence of AceA, fungi are unable to regulate exposure to host-derived copper through copper transporters. *In vivo*, AceA, induction of the copper exporter CrpA, or depression of ROS-mediated host defense is necessary for virulence in cortisone-treated mice [149]. Other metal metabolism pathways are also important, including the SchA kinase, which is upregulated during states of iron starvation. SchA was found to play a role in iron assimilation (among other functions), and in its absence, *A. fumigatus* is avirulent in a neutropenic mouse model of IA [150].

A. fumigatus growth in the lung

Beyond fungal metabolism and stress adaptation, a number of fungal genes are important for hyphal growth and pathogenesis in the respiratory tract. Myosins have critical roles in the growth of *Aspergillus* hyphae and the pathogenesis of IA, with a requirement for MyoE in vesicle trafficking to the Spitzenkörper (i.e., cluster of transport vesicles located at growing hyphal tips) and for MyoB in regulating normal formation of septa. Although MyoB is dispensible for growth, MyoB-deficient conidia exhibit decreased viability and the deletion mutants are hypovirulent in a cyclophosphamide/corticosteroid mouse model of IA. Similarly, hyphal extension

requires MyoE function, and as a result, MyoE deletion mutants are also hypovirulent [151].

Aspergillus hyphae are able to penetrate the epithelial barrier by tunneling into the epithelial cells without impacting tissue integrity [152]. In addition, *A. fumigatus* is able to co-opt membrane proteins to enable uptake by epithelial and endothelial cells. The thaumatin-like protein CalA interacts with host cell integrins to enable its invasion by inducing fungal endocytosis into non-hematopoietic cells. In immunocompromised mice, CalA deletion mutant infection results in longer survival, reduced burden, and less tissue invasion. This phenotype can be recapitulated with wild-type *A. fumigatus* strains by blocking CalA with an antibody [153].

Examples of *A. fumigatus* immune evasion

A. fumigatus completes its life cycle outside the human host, and as stated above, virulence attributes reflect adaptations to its natural habitat (i.e., decaying organic matter). Some of these attributes enable the fungus to counter and evade host defense mechanisms in the mammalian respiratory tract.

Melanin removal and exposure of immune reactive cell wall polysaccharides during germination precede the induction of LAP. In accordance with this model, the hypovirulent phenotype of amelanotic conidia is reversed when LAP is impaired [105]. Deletion of the fungal cell wall protein CcpA enhances the immunoreactivity of swollen conidia, resulting in attenuated virulence in a murine corticosteroid model of IA. This finding suggests that CcpA, like hydrophobins in resting conidia, conceals the surface expression of immunoreactive polysaccharides [154].

Evasive hyphal branching represents a hyphal response to noxious stimuli that may serve as an immune evasive mechanism in human tissues. *In vitro*, *A. fumigatus* hyphae generate evasive branches upon interaction with neutrophils. The *in vivo* implications of this growth phenotype remain unclear [155].

The *A. fumigatus* metalloprotease Mep1p is released upon fungal interactions with collagen and can degrade various complement components. *In vitro*, Mep1p reduces complement deposition on conidia and the ensuing neutrophil phagocytosis. However, in a cyclophosphamide model of IA, Mep1-deficient conidia displayed no difference in virulence than wild-type conidia, suggesting that Mep1p-dependent effects on host complement components do not alter disease outcomes [156].

Conclusion

IA has emerged as an unmet medical need due to advances in medical technologies that have increased the at-risk population. Despite improvements to the standard of care and the use of triazole drugs, IA mortality remains unacceptably high. Moreover, azole-resistant *Aspergillus* isolates are increasing in prevalence in the clinic, likely due to agricultural use of azole drugs [157]. Recent advances in understanding the molecular and cellular determinants of antifungal immunity and the tissue-specific and metabolic determinants of fungal pathogenesis have revealed new targets for antifungal therapeutic strategies, including small molecules, which target the cell wall, the mitochondria, and pyrimidine biosynthesis, and even Dectin-1 directed CAR-T cells [158]. Future research will need to emphasize clinical translation of these results to improve IA outcomes in vulnerable patient groups.

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Abbreviations used:
IA, invasive aspergillosis; CGD, chronic granulatomous disease; DHN, 1,8-dihydroxynapthalene; CR3, complement receptor 3; NETs, neutrophil extracellular traps; BTK, Bruton's tyrosine kinase; BCR, B-cell receptor; IFN, interferon; GM-CSF, granulocyte−macrophage colony-stimulating factor; pDCs, plasmacytoid dendritic cells; NK, natural killer; A-RCD, apoptosis-like regulated cell death; Syk, spleen tyrosine kinase.

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