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Host Response to Pulmonary Fungal Infections: a Highlight on Cell-Driven Immunity to *Cryptococcus* Species and *Aspergillus fumigatus*

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Abstract

Purpose of Review In this article, we provide a summary of recent findings in the field of host responses to fungal infections caused by the environmental fungi *Aspergillus fumigatus* and *Cryptococcus* species. Key myeloid and lymphoid cells that are important in antifungal responses are discussed. The primary leukocytes discussed are macrophages, monocytes, dendritic cells, neutrophils, natural killer cells, and T cells, all of which play roles in conferring protective immunity to these opportunistic pathogens.

Recent Findings An immunocompromised status is the primary risk factor for *Aspergillus*- and *Cryptococcus*-induced morbidities and mortalities, despite the use of current antifungal therapeutics. Research in the past several years has revealed that a variety of immune cell types play critical roles in mounting protection in healthy individuals. The essential effector responses involved in anti-*Aspergillus* and anti-*Cryptococcus* immunities differ in several aspects, despite the fact that both are fungi that in humans primarily colonize the pulmonary tissue. Alveolar macrophages and neutrophils comprise the first line of defense that engulfs and eliminates fungi, primarily through the release of free radicals. Monocytes and dendritic cells are the immune sentinels that not only directly eliminate fungi, but also modulate the functions of other immune cells and activate fungus-specific T cells.

Summary Increased prevalence of immunosuppressive drugs and incidence of diseases such as HIV/AIDS have led to greater numbers of immune-incompetent hosts who are at risk for fatally invasive *Aspergillus* or *Cyrptococcus* infections. Elucidating the mechanisms and key immune effectors that combat these infections is crucial for the development of future therapies.

This article is part of the Topical Collection on *Immunology and Inflammation*

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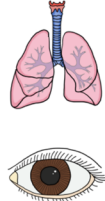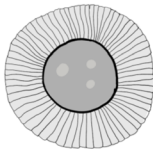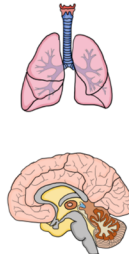
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Introduction

The incidence of fungal infections has increased over several decades due to the development of new clinical interventions (therapies or drugs) or diseases (such as HIV) that lead to suppressed host immunity. Patients with impaired immune function can develop life-threatening infections with fungal pathogens that would otherwise be controlled (Fig. 1) [1, 2]. Hence, advances in modern medicine have unintentionally created a niche for fungal pathogens. An additional complicating factor is the rise of drug-resistant fungal strains [2–5]. In aggregate, fungal infections affect billions of people per year, worldwide, and lead to the death of at least as many people as does tuberculosis or malaria [6–12]. Despite the detrimental impact of fungal infections to human health, there are no approved vaccines for any fungal pathogen [1, 13]. The successful development of antifungal vaccines could help

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Fig. 1 Fungal pathogens have the potential to cause disease in a multitude of tissues. Commensal or environmental fungi can become disseminated infections in immunocompromised individuals. *Aspergillus fumigatus* and *Cryptococcus* species are two of the most common and clinically relevant environmental fungi capable of causing severe pulmonary disease.

Fungus	Site of infection	Protective Cells	Protective Cytokines
A. fumigatus		Monocytes Macrophages Dendritic cells Neutrophils	GM-CSF IL-1 IL-17 TNFα
C. neoformans		Monocytes Dendritic cells CD4+ T cells (Th1 and Tregs) CD8+ T cells	IL-12 IFN-γ TNFα IL-6 Type I IFN GM-CSF
		Macrophages Neutrophils CD4+ Th1 cells	

prevent the high numbers of life-threatening infections as well as potentially benefit the millions of people that contract superficial infections or fungal-related allergies.

Research efforts to gain new knowledge on the mechanisms utilized by the host to combat fungal infections and the mechanisms that fungi utilize to subvert them will likely facilitate the future development of much needed new therapies. A better understanding of how the host responds to control fungal pathogens is likely to identify host factors that can be exploited in the development of immune-based therapies. The use of combinatorial therapies where immune-based therapies and novel antifungal drugs work alongside to fight fungal infections will be critical to help improve patient outcomes in the future [13].

Given the importance of host immune status to the susceptibility to fungal infections, in this review, we summarize our current understanding of relevant mechanisms of host immunity against fungi. We will focus our discussion to the main cell types involved in the pulmonary immune responses to two major fungal pathogens: *Aspergillus fumigatus* and the *Cryptococcus* species. These two pathogens account for the vast majority of the fungal infections worldwide, particularly in immunocompromised hosts [1, 13–16]. The environmental fungi *A. fumigatus* and *Cryptococcus neoformans* are widely distributed around the world. Given the high prevalence of these fungi, it has been estimated that humans are constantly exposed to these fungal pathogens [17, 18]. In the context of normal immune status, this exposure has no detrimental consequences, but if host integrity is compromised, these otherwise minor threats can lead to major life-threatening infections. In this review, we will summarize recent advances in our understanding of the role of specific immune cells in the control of infection with these two clinically important fungal species, with a primary focus on innate immunity. For more in-depth discussions on other relevant aspects of host immunity to fungi, please see recent reviews [19, 20].

Myeloid Responses

Macrophages

Tissue-resident macrophages are a group of heterogeneous immune cells with a range of utilities including homeostatic functions, immune surveillance, and resolution of infection and inflammation. In the lung, alveolar macrophages are specialized tissue macrophages that reside between the airway epithelium and pulmonary vasculature [21, 22]. The functional requirements of macrophages vary from clearance of cellular debris [23] to phagocytosis and elimination of pathogens [24–26]. As a result, the plasticity of tissue-resident macrophages is important to maintain a balance of the inflammatory environment and prevent responses to non-pathogenic antigens. At the steady state, alveolar macrophages are poor antigen presenters and can induce T cell tolerance to harmless stimuli [27, 28]. They normally exist in a non-inflammatory state with poor phagocytic capability [29–31]. However, the engagement of multiple pattern recognition receptors (PRRs) and the disruption of the structural environment can initiate a more inflammatory state. In this activated inflammatory form, macrophages have greater phagocytic ability and more efficient oxidative burst and secrete pro-inflammatory cytokines to override the inhibited T cell responses [30, 32, 33]. In addition to alveolar macrophages, monocyte-derived "classically" and "alternatively" activated macrophages are also

key mediators of host defense and clearance of fungal infection in the lung [34].

Alveolar macrophages are the main phagocytic cells encountered early in *Aspergillus* infection [35, 36]. Past studies have shown that *Aspergillus* conidia are engulfed by alveolar macrophages and killed by maturation and acidification of the phagolysosome [36, 37]. The importance of monocyte-derived classically versus alternatively activated macrophages in progressing the pathology of *Aspergillus* infections is somewhat debatable. One study has shown that alternatively activated macrophages, characterized by fungus-induced expression of arginase-1 and surface expression of CD206, protected against *Aspergillus* infection [35]. However, PU.1, a transcriptional regulator of alternative polarization of macrophages, has been implicated in the pathological outcome of *Aspergillus*-induced asthmatic airway inflammation [38, 39]. Moreover, classical macrophages are a potent source of reactive oxygen species and can promote Th1 response [40, 41], both of which have been shown by our studies and others to be helpful in the control of *Aspergillus* infection [42–48]. However, as it is becoming more apparent that the distinctions between "classical" and "alternative" are fluid characterizations, it is likely that both macrophage activation states are functionally required for mounting a robust defense against *Aspergillus*.

Similarly, the subtype diversification of macrophages appears to be highly plastic and adaptable to external signals during cryptococcal infection [49]. A recent study demonstrated that macrophage-dependent STAT1 signaling was critical for limiting uncontrolled inflammation in the lung, regulating an optimal Th1/Th2 response, and NO-dependent protection against H99, a virulent strain of *C. neoformans* [50]. In contrast to *Aspergillus*, CD4$^+$ T cells are thought to be the most critical component of intact immunity against *Cryptococcus* [51]. However, a recent study found that even in the presence of sufficient T cell recruitment, a skewed M2 macrophage polarization led to failure to appropriately phagocytose and eliminate fungal cells, resulting in cryptococcal meningitis in otherwise immunocompetent individuals [52].

Recently, a study also reported the importance of a macrophage-scavenging receptor, MARCO (macrophage receptor with collagenous structure), in modulating the immune response to pulmonary *Cryptococcal* infection. Expression of MARCO was required for sufficient production of protective cytokines including IFN-γ and IL-12 [53]. In addition, the upregulation of CCR2 chemokine ligands was dependent on MARCO expression; mice lacking MARCO had dampened recruitment and classical activation of monocyte-derived dendritic cells (moDCs) [53]. Finally, interactions between fungal cells and pulmonary macrophages and moDCs were significantly reduced in MARCO$^{-/-}$ cells, resulting in diminished phagocytosis [53]. Altogether, these findings indicate the crucial role of MARCO-expressing macrophages in shaping the early immune response to *C. neoformans*.

The activation of macrophages by *A. fumigatus* occurs through the engagement of TLRs 2 and 4 [54], and C-type lectin receptors (CLRs) [55, 56]. The stimulation of TLRs as well as Fc receptors in macrophages triggers a non-canonical autophagy pathway driven by LC3, known as LC3-associated phagocytosis (LAP) [57, 58]. The engulfment of *Aspergillus* induces formation of a LAPosome which requires the class III PI3(K)-associated protein Rubicon [59]. Mice that either lacked Rubicon or had macrophages with defective LAP machinery were unable to properly clear *Aspergillus* spores and had greater pulmonary inflammation [59]. Impaired autophagy is exhibited in patients and murine models of chronic granulomatous disease (CGD), a disorder characterized by a lack of intact NADPH-oxidase activity [60, 61]. Patients with CGD are at greater risk of various infections, including invasive pulmonary aspergillosis (IPA) [62, 63]. Both monocytes from CGD patients and lung macrophages from $p47^{phox-/-}$ mice displayed impaired expression of death-associated protein kinase 1 (DAPK1), a component of LAP [64]. The expression of DAPK1 in wild-type mice was upregulated 4 hours after *Aspergillus* infection and was dependent on IFN-γ expression. The treatment of *Aspergillus*-infected $p47^{phox-/-}$ mice with recombinant IFN-γ restored DAPK1 expression in lung macrophages and controlled fungal growth [64]. LC3-independent autophagy appears to be more important during cryptococcal infection. The disruption of autophagy-related 5 (ATG5) protein in J774.16 cells in vitro inhibited the phagocytosis and restriction of *C. neoformans* cells. Bone marrow macrophages from $atg5^{-/-}$ mice pre-activated with IFN-γ and LPS were more permissible to *C. neoformans* growth. On the other hand, unactivated $atg5^{-/-}$ macrophages exhibited greater fungicidal activity. These observations suggest a complex role for autophagy in defense against cryptococcal infection, dependent on macrophage type and activation state [65]. Together, these findings demonstrate the importance of the linked macrophage phagocytosis and autophagy pathways in immunity to fungal infections.

Recently, a fucose-binding lectin (FleA) on the surface of *Aspergillus* has been shown to strongly bind to surface receptors on macrophages [66]. This lectin is one of many pathogen-associated molecular patterns (PAMPs) that become accessible to macrophage CLRs and mannose receptors during the germination of *A. fumigatus* conidia [55, 66, 67]. One of the most prevalent surface PAMPs on swollen *A. fumigatus* conidia is β-glucan [55, 67, 68]. Its cognate receptor, dectin-1, is capable of triggering calcineurin- nuclear factor of activated T cell (NFAT) binding in macrophages [69]. Additionally, a dectin-1- and MyD88-independent pathway has recently been found to mediate phagocytosis-dependent immune responses in macrophages. The engagement of TLR9 by *Aspergillus* spores leads to signaling via Bruton's tyrosine kinase (BTK) [70]. Signaling through either pathway in turn drives downstream calcineurin-NFAT transcription responses in

macrophages, which, in collaboration with NFκB, induces increased expression of TNF-α and IL-2 [70, 71]. Organ transplant patients are often treated with calcineurin inhibitors, which impair this signaling pathway and innate pulmonary responses [72, 73]. Patients treated with calcineurin inhibitors such as cyclosporine A and tacrolimus also displayed impairment in pentraxin-3 function, which was linked with increased susceptibility to aspergillosis and severe invasive aspergillosis (IA)-induced morbidity [71]. Furthermore, a recent report demonstrated that DAP12, an adaptor protein for multiple PRRs, acts as limits pulmonary inflammation during *C. neoformans* infection. Bone marrow-derived macrophages from DAP12$^{-/-}$ mice displayed improved antifungal function, including improved phagocytosis and increased production of pro-inflammatory cytokines such as TNF [74]. Although DAP12$^{-/-}$ mice eventually succumb to infection [74], this protein may be one potential target of combinatorial therapeutics.

Monocytes and Dendritic Cells

CCR2$^+$, inflammatory monocytes, is also an essential innate cell in the defense against pulmonary infection with *A. fumigatus* [75–77]. The contributions of inflammatory monocytes to antifungal immunity are multifactorial and involve the direct eradication of fungal conidia and the production of protective cytokines. Inflammatory CCR2$^+$ monocytes also assist in the clearance of cryptococcal infection [78, 79]. However, there is some debate whether monocytes may actually propagate *C. neoformans* infections. Studies have shown that *Cryptococcus* yeast phagocytosed by macrophages and monocytes may employ a "Trojan horse" mechanism to pass the blood brain barrier, leading to meningoencephalitis [80, 81].

In addition to direct killing of fungi, an intact CCR2$^+$ monocyte compartment is required for a robust antifungal repsonse by neutrophils. It is likely that monocyte-derived factors are required for the activation of optimal neutrophil responses [75]. Indeed, CCR2$^+$ cell ablation leads to diminished expression of IL-1α and CXCL1 24 hours after challenge with *Aspergillus* conidia and subsequent reduction in early neutrophil recruitment [82]. Recent studies from our lab have further identified CCR2$^+$ monocyte-derived type I interferon (IFN) as an important mechanism of monocyte-dependent regulation of antifungal neutrophil activation [83]. We uncovered an essential role for type I and III IFNs as regulators of neutrophil antifungal activity where both IFNs were required for the efficient production of reactive oxygen species (ROS) [83]. Optimal production of type III IFN required early production of type I IFN by CCR2$^+$ monocytes [83]. Thus, CCR2$^+$ monocytes orchestrate antifungal defense against IA by initiating a coordinated IFN response that further promotes antifungal neutrophil activation [83]. Future studies are likely

to further identify monocyte-dependent mechanisms that shape antifungal immunity.

Dendritic cells (DCs) are specialized innate cells originating from hematopoietic progenitors. These highly phagocytic cells are exceptionally proficient in antigen processing and presentation [84]. Subsets of DCs include plasmacytoid dendritic cells (pDCs), classical dendritic cells (cDCs), and moDCs. An in-depth discussion of dendritic cell lineage and diversification can be found in several reviews [84–87].

moDCs, also known as inflammatory DCs, arise from bone marrow-derived CCR2$^+$ monocytes. During infection, CCR2$^+$ monocytes migrate to the site of inflammation and differentiate into inflammatory DCs [88, 89]. Upon *A. fumigatus* infection, CCR2$^+$ monocytes are rapidly recruited to the lung and give rise to moDCs that are critical for the activation of fungus-specific CD4 T cells [90]. Upon *C. neoformans* infection, the lack of CCR2$^+$ cells resulted in diminished recruitment of cDCs to the lung and subsequently impaired Th1 response [91]. Bone marrow-derived DCs primed with a vaccine strain of *Cryptococcus gatii* were able to confer protective immunity in mice challenged intratracheally with the strain R265 in an IFN-γ-dependent manner [92]. Together, these studies demonstrate the importance of DCs during *Cryptococcus* infection. Future investigations will need to dissect whether dendritic cells, similar to monocytes, may also play a pathogenic role in the dissemination of *Cryptococcus* and the potential mechanisms involved in maintaining a sufficiently protective innate response.

In response to *Aspergillus,* CCR2$^+$ moDCs have been shown to directly engulf and kill conidia. In their absence, mice succumbed to IPA [75]. Furthermore, pDCs were found to display anti-*Aspergillus* response in a dectin-2-dependent manner. Upon hyphal stimulation, pDCs secrete IFN-α and TNF-α and appear to form pDC extracellular traps [93] that likely confer protection against germinating *Aspergillus* spores.

Despite in vitro findings that suggest that *C. neoformans* is a poor activator of pDCs [94], a recent report demonstrated that pDCs can phagocytose *C. neoformans* cells and inhibit fungal growth. This function was dependent on dectin-3 expression [95]. However, dectin-3 is not required for immunity to *C. neoformans*, suggesting that pDCs might employ other innate receptors in the defense against *C.neoformans* or that other DC subsets can compensate for pDC function in protection against pulmonary cryptococcal infection in vivo [96].

Current findings also suggest that monocytes and dendritic cells are critical not only for the direct elimination of *A. fumigatus* spores, but also in coordinating the response of other effector cells [97]. Notably, in the absence of CCR2$^+$ inflammatory monocytes and moDCs, the conidiacidal activity of neutrophils was diminished [75, 83]. Dendritic cells are also implicated in the control of the balance between protective and pathogenic inflammations. *Aspergillus*-induced IL-2

and IL-23 secreted by classical CD103^{+} DCs maintain an optimal Th17 response. In the absence of IL-2, there is unrestricted IL-23-induced Th17 polarization, leading to fatal hyper-inflammation [98].

Dendritic cells recognize *A. fumigatus* spores via the C-type lectin, dectin-1. When dectin-1 was blocked on human cDCs, there was diminished expression of the T cell co-stimulatory molecules CD40, CD80, and HLA-DR, as well as impaired production of TNF-α and IL-1β [99]. The formation of a phagocytic synapse enables the CLR dectin-1 to recognize of β-glucan on the surface of *Aspergillus* [100]. Phagocytosis leads to lysosomal processing of the fungi and expression of CD86 and CD83 co-stimulatory markers. Subsequently, mature DCs become efficient antigen-presenting cells and activators of T cell responses. In aggregate, these findings indicate that β-glucan-dependent recognition of *Aspergillus* is critical for efficient dendritic cell function.

Neutrophils

Neutrophils are the early responders to a variety of pathogens, including bacteria [101, 102], viruses [103, 104], and fungi [75, 105, 106]. Early in their discovery, neutrophils were thought to be simple, short-lived white blood cells that destroyed foreign intruders by "suicide" methods [107]. However, in the last couple of decades, it has become apparent that neutrophils possess several sophisticated mechanisms for detecting and eliminating pathogens. Detailed discussions on the functions of neutrophils during steady state and in inflammatory environments can be found in the following reviews [108–111].

Neutrophils have long been characterized as important effectors in defense against *A. fumigatus*. Neutropenia following hematopoietic stem cell transplantation, as well as immunotherapies that suppress neutrophil function, is a major risk factor for IPA [112]. Neutrophils inhibit invasive fungal disease through the production of ROS and release of proteases via degranulation [113, 114]. In addition, neutrophils extrude nuclear extracellular traps (NETs) that contain peptides including elastases and calprotectin that exert antimicrobial functions in a process known as NETosis [115–117]. Signals transduced by both the MyD88 and CARD9 adaptors were shown to be essential for neutrophil-mediated antifungal responses. Defects in either molecule caused delayed or interrupted the expression of neutrophil chemokines CXCL1 and CXCL5, leading to insufficient neutrophil recruitment to the lung, resulting in fatality [56].

During *Aspergillus* infection, neutrophils are early recruits to the lung. In immunocompetent hosts, neutrophils form complexes with conidia that possess NADPH oxidase activity, thereby inhibiting germination [118]. Using in silico modeling, a recent report demonstrated that 6–17 hours after initial exposure, neutrophil- rather than macrophage-mediated killing of *Aspergillus* is the dominant conidiacidal response [119]. In addition, iron sequestration by lactoferrin secreted in neutrophilic granules is involved in restricting the initial germination of conidia [120, 121]. Recognition of conidia by neutrophils was found to depend on the integrin CR3. In contrast, detection of hyphae relies on FcγR stimulation by opsonized fungi [121].

Murine models have demonstrated that neutrophils are also essential in limiting hyphal growth in both pulmonary [75, 122, 123] and corneal [124] models of IPA. Neutrophils employ a variety of extracellular mechanisms in order to regulate unrestricted germination. The primary mechanism for destruction of hyphae is mediated by toxic superoxides produced by NADPH oxidase and myeloperoxidase [121]. It has been suggested that ROS derived from longer lived (> 10 hours) neutrophils can act as a sensor of microbe size to induce cytokine production, driving further recruitment of neutrophils and subsequent clustering at the site of hyphal formations [125]. This model challenges the classic paradigm that neutrophils are only short-lived cells that primarily function through apoptotic mechanisms. Non-oxidative mechanisms also play a role in clearing *Aspergillus* hyphae. The zinc- and manganese-chelating protein, calprotectin, has been shown to inhibit hyphae in a murine model of fungal keratitis [126].

Neutrophils also employ phagocytic mechanisms for killing earlier morphotypes of *Aspergillus*. Complement-driven phagocytosis and killing of swollen conidia and germ tubes by neutrophils are dependent on opsonization by the serum components C3b and IgG [127]. Leukocyte-intrinsic granulocyte macrophage colony-stimulating factor (GM-CSF) is also required to kill phagocytosed spores by enhancing NADPH oxidase-dependent conidiacidal activity [128]. Not only do neutrophils eradicate *Aspergillus* through direct mechanisms, but they also interact and synergize with other leukocytes. Importantly, this includes inflammatory monocytes and their derivative cells [75].

As one of the first responders in the lung, neutrophils also have a role in pulmonary *Cryptococcal* infection. Using live cell imaging, recent studies demonstrated that neutrophils actively migrate towards and rapidly phagocytose *C. neoformans* cells [129]. The complement factors C5a-C5aR enhance phagocytosis of *C. neoformans* by stimulating neutrophil upregulation of CD11b. In addition, C5a-C5aR signaling mediates chemotaxis of neutrophils towards *Cryptococci* via the p38 MAPK pathway [129]. Neutrophils were also shown to be critical in the clearance of disseminated *C. neoformans* in brain vasculature [130]. However, other studies have suggested the potential deleterious role of neutrophils during *Cryptococcal* infection. Depletion of neutrophils with anti-GR1 led to extended survival and reduced inflammatory lung damage without reduction in early fungal burden [131]. Moreover, mice with genetically induced

neutrophilia seem to have increased susceptibility to pulmonary cryptococcal disease [132]. Further studies are needed to elucidate the implication of enhanced neutrophil recruitment during cryptococcal meningoencephalitis.

Lymphoid Responses

Natural Killer Cells

Natural killer (NK) cells are innate effector cells of lymphoid origin. They were originally found to be important in surveilling and exhibiting cytotoxic activity against tumor cells. They possess specialized receptors that allow them to distinguish healthy cells from those missing or expressing aberrant "self" molecules [133–137]. However, NK cells also have critical roles in immunity against external pathogens. There is strong evidence for activity of NK cells in control of viral and bacterial infections [138–141]. NK cells are one of the resident cells in the lung. Under steady state, NK cells in the lung are quiescent, tightly regulated by TGF-β and prostaglandins released by alveolar macrophages, and have severely diminished cytotoxic capability [142–144]. Upon stimulation with pro-inflammatory cytokines such as type I IFNs, IL-12, and IL-8, NK cells regain their function [142, 145–147]. NK cells enact their defensive function through the expression of IFN-γ as well as the degranulation of perforin and other cytotoxic peptides such as serine proteases [142, 145, 147, 148]. The various functions and roles of NK cells are addressed in detail in several reviews [133, 138, 149–151]. Here, we briefly discuss a few functions relevant to *Aspergillus* and *Cryptococcus*.

There is some evidence for the importance of NK cells in defense against IPA. One study has shown activity of human NK cells against *A. fumigatus* hyphae, but not conidia [152]. Another group's findings suggest that human NK cells can kill *Aspergillus* upon activation by germlings and that this appears to occur through a perforin-independent manner [153]. In a model of neutropenia, NK cells were found to mediate protection from IA suggesting that in the absence of an essential antifungal cell (neutrophils), NK cells can help protect the host against IA [154]. Whether NK cells play essential roles in defense in the presence of neutrophils is unclear. We find that RγC$^{-/-}$ mice, which lack the entire lymphoid lineage (including NK cells), retain protective immunity to *A. fumigatus* [75, 83]. Thus, in the context of intact myeloid cell responses, the role of NK cells and other lymphoid lineage-derived cells appears to be secondary.

However, several studies have shown corroborating evidence for direct binding and cytotoxic function of NK cells against *Cryptococcus* [155–158]. The NKp30 receptor is responsible for anticryptococcal activity by mediating direct fungal binding and perforin release. Interestingly, NK cells

from HIV patients had defective polarization of perforin granules and diminished NKp30 expression [159]; treatment with IL-12 restored microbiocidal activity [160]. This is likely caused by augmented NK cell cytotoxicity due to increased expression of activation receptors, including NKp30, and enhanced the release of granule-derived proteases [159, 160].

Primary human NK cells were found to have greater anticryptococcal activity in acidic, rather than physiological, pH [161]. This is unsurprising as NK cells are commonly associated with surveilling in tumor microenvironments [151]. Furthermore, NK cells were also found to be closely associated with *C. gattii* cells within a cryptococcoma [161]. Altogether, these findings suggest that NK cells provide protective functions against *Cryptococcus* cells within hypoxic milieus where other immune cells may not be present or as persistently active.

T Cells

The diversity and plasticity of CD4$^+$ T helper cell subsets are critical for mounting robust immune responses to pathogenic challenge. The roles of the various subtypes are described in detail in other reviews [162, 163]; we discuss some functions relevant to *Aspergillus* and *Cryptococcus*.

Following the innate response phase, *Aspergillus*-specific T cells are rapidly primed by antigen-presenting cells in the draining lymph node [164, 165]. The resulting CD4$^+$ T cell response is diverse and consists predominantly of Th1 and a smaller population of Th17 effectors [43, 164]. Dectin-1-mediated signals suppress Th1 and IFN-γ responses and enables Th17 differentiation [43]. Th1 cells are associated with the pro-inflammatory cytokines IFN-γ, IL-12, and TNF-α that not only further shape the CD4$^+$ T cell response, but also enhance the phagocytic and direct antifungal functions of macrophages and monocytes [166]. Immunotherapy with recombinant IFN-γ in patients with invasive fungal infections partially restored cell-mediated antifungal responses [167]. Th17-derived IL-17 has also been found to play a significant role in conferring protective immunity in *Aspergillus*-driven fungal keratitis [124].

Exaggerated Th2 responses induced by *Aspergillus* have been associated with pathogenic lung inflammation and allergic disease [168, 169]. Increased chitin-to-β-glucan ratio due to prophylactic caspofungin, an inhibitor of β-glucan synthesis, has been associated with a type 2 cytokine profile and increased eosinophil-induced airway inflammation in mice [170–173]. Airway eosinophilia in response to a chitin-overexpressing environmental isolate was considerably diminished in mice lacking γδ$^+$ T cells [174]. Furthermore, TCRγδ$^{-/-}$ mice exhibited greater protective response to *Aspergillus*, resulting in decreased fungal burden and pulmonary eosinophilia [173]. These observations indicate the importance of cell wall components in shaping the immune

response to *A. fumigatus* and identify $\gamma\delta^+$ T cells as potential regulators of pathogenic lung inflammation. During acute cryptococcal infection, $\gamma\delta^+$ T cells accumulate in the lung [175]; however, their exact role remains unclear. One study reported a protective function of IL-17-producing $\gamma\delta^+$ T cells in a neutropenic model of cryptococcal infection [176], while another observed enhanced fungal clearance and beneficial Th1 response in TCR$\gamma\delta^{-/-}$ mice [175].

CD4$^+$ T cells are fundamental in immunity against *Cryptococcus*. This is illustrated by the high incidence of cryptococcosis in patients suffering from HIV/AIDS [177–179]. Impaired clearance of *Cryptococcus* is associated with significantly reduced CD4$^+$ and CD8$^+$ T cell numbers [51, 180]. Both CD4$^+$ and CD8$^+$ T cells contribute to response to pulmonary *Cryptococcus;* their respective roles are discussed in detail in previous reviews [180, 181]. CD4$^+$ T cells play a dominant role in chemotaxis and activation of macrophages and granulocytes to the lung [182, 183]. *Cryptococcus*-specific CD4$^+$ T cells are important sources of macrophage inflammatory proteins 1α, IFN-γ, and TNF-α [180, 184–186]. In a recent clinical study of patients with HIV-associated cryptococcal meningitis, 25% of individuals with low IFN-γ and TNF-α- responses succumbed to the infection, while those with intact signaling survived [184]. Another report found that in HIV/AIDS patients, a protective immune response was associated with the presence of IL-17 and IFN-γ. Patients with even a minimal *Cryptococcus*-specific Th1 profile had faster fungal clearance from the CNS [187]. While *C. neoformans* primarily affects immunocompromised individuals, *C. gatii* is more commonly reported in immunocompetent hosts [188]. This species is suspected to be capable of dampening the Th1/Th17 response, thereby increasing the susceptibility of an otherwise healthy host to systemic cryptococcosis [189]. In aggregate, these studies highlight the relevance of CD4$^+$ T cells, especially Th1-derived IFN-γ, as essential contributors to protective immunity against *Cryptococcus*.

Concluding Remarks

Fungal pathogens are major causes of morbidity and mortality in immunocompromised patients despite the use of current antifungal chemotherapeutics. *Cryptococcus* species and *A. fumigatus* comprise some of the most common fungal pathogens that humans can be exposed to. Over the last several years, research efforts have begun unraveling the cellular mechanisms that mediate protective immunity against these species. Understanding these mechanisms is key for the development of more effective therapeutics in the future. Ongoing research in this area continues to provide exciting new knowledge on relevant mechanisms of antifungal defense that could be developed into effective immunotherapies.

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