Innate Lung Defense during Invasive Aspergillosis: New Mechanisms

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Innate immunity · *Aspergillus fumigatus* · Pattern recognition receptors · Cytokines

Abstract

Invasive aspergillosis (IA) is one of the most difficult to treat and, consequently, one of the most lethal fungal infections known to man. Continued use of immunosuppressive agents during chemotherapy and organ transplantation often leads to the development of neutropenia, the primary risk factor for IA. However, IA is also becoming more appreciated in chronic diseases associated with corticosteroid therapy. The innate immune response to *Aspergillus fumigatus*, the primary agent in IA, plays a pivotal role in the recognition and elimination of organisms from the pulmonary system. This review highlights recent findings about innate host defense mechanisms, including novel aspects of innate cellular immunity and pathogen recognition, and the inflammatory mediators that control infection with *A. fumigatus*.

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Introduction

Concerns are mounting over the rise in invasive fungal infections (IFIs) caused by *Candida*, *Cryptococcus*, and *Aspergillus* species over the last several decades due to modern medical interventions, such as immunosuppressive drugs. IFI caused by *Aspergillus fumigatus*, i.e., inva-

sive aspergillosis (IA), remains one of the most lethal human infectious diseases. Development of IA may be the result of multiple predisposing factors, but immunosuppression leading to neutropenia remains the predominant risk factor [1, 2]. Solid-organ transplantation (approx. 30,000/year in the USA) and hematological malignancy, primarily acute myeloid leukemia and acute lymphoblastic leukemia requiring stem-cell transplantation (approx. 20,000/year in the USA), are the greatest risk factors for the development of IA [3]. The most recent data from the Transplant-Associated Infections Surveillance Network reflect an IFI incidence rate of 7% in both solid-organ [4] (19% due to IA) and stem-cell [5] (43% due to IA) transplantation. IA is particularly common in lung transplantation, where it is associated with >70% of IFIs [6]. ICU patients represent a newly appreciated and significant at-risk population for IA. These patients often present with exacerbations of chronic obstructive pulmonary disease, alcoholic liver cirrhosis, and solid cancers. High-dose corticosteroid usage is a frequent comorbidity, and in-hospital mortality can range from 46 to 79% [7–10].

Cellular Effectors

Neutrophils

Neutrophils have long been appreciated as the essential cell type for eliminating *A. fumigatus*, and neutropenia remains the primary risk factor for developing inva-

sive aspergillosis. Consequently, neutrophils continue to be a focus of investigation. Previous studies suggested that neutrophils were primarily effective against hyphae rather than conidia [11], although recent data challenge this assertion. Investigating neutrophils from individuals with a variety of defects in innate immune genes has revealed that neutrophil-mediated recognition of conidia requires CD11b/CD18 (e.g., complement receptor 3) rather than Dectin-1. Conversely, hyphal recognition requires antibody-mediated opsonization and Fc γ receptors [12]. Mechanistically, the killing of *A. fumigatus* hyphae by neutrophils requires both NADPH oxidase and myeloperoxidase, but not the formation of neutrophil extracellular traps (NETs). In contrast, the killing of conidia is achieved by nonoxidative lactoferrin-mediated iron sequestration. An additional study has recently examined interactions between human neutrophils and *A. fumigatus* conidia and hyphae in the presence of various chemoattractants and neutrophil activators [13]. Using a nanoliter culture well system, this study demonstrated that less than a third of conidia form hyphae when incubated with pure neutrophils, an amount that continues to decrease when the cocultures contain a uniform amount of leukotriene B₄, but not fMLP. In contrast, using a chemotaxis chamber, unprimed neutrophils reduce hyphal formation by approximately 50% whereas the addition of fMLP or leukotriene B₄ gradients increases neutrophil chemotaxis and a more rapid phagocytosis of conidia, with leukotriene B₄ demonstrating superior neutrophil priming compared to fMLP. Neutrophils from patients undergoing immunosuppressive therapy are less effective at limiting hyphal transformation and appear less responsive to fMLP stimulation.

Neutrophils express multiple pattern recognition receptors (PRRs) involved in *A. fumigatus* recognition, including the β -glucan receptors Dectin-1 and Mac-1 (CR3). Although studied with the fungal particle zymosan or the pathogenic fungal organism *Candida albicans*, it was recently reported that Dectin-1 and Mac-1 induced the expression of protein kinase C γ in neutrophils [14]. Neutrophils from mice deficient in protein kinase C γ have impaired reactive oxygen species (ROS) generation, granule content release, cytokine production, and killing of *C. albicans*. Upon fungal exposure, protein kinase C γ mice were unable to clear a systemic (*C. albicans*) or pulmonary (*A. fumigatus*) challenge, indicating that protein kinase C γ has a central role in regulating the degradative and microbicidal properties of neutrophils required for fungal clearance [14].

Previous studies documented an interesting, yet unexpected role for neutrophils in regulating the number of inflammatory dendritic cells (DCs) in the lung during *A. fumigatus* exposure [15]. A recent follow-up to this study determined that DCs recruited in the absence of neutrophils display a more immature phenotype [16]. In fact, when neutrophils are cocultured with DCs, an increase in costimulatory molecule expression occurs, a process that requires the C-type lectin receptor, DC-SIGN. Another intriguing study recently characterized the spatiotemporal recruitment (examined at 4, 16, and 40 h after challenge) of neutrophils and other innate cells to the lung in 2 different murine models of immunosuppression-associated IA, i.e., mice receiving cyclophosphamide + cortisone treatment (CCT) or corticosteroid treatment (CT) [17]. The CCT model demonstrated earlier mortality (100% within 4 days) and showed a profound reduction in the neutrophil and macrophage populations in the lung with 4 h of exposure. In contrast, despite demonstrating 100% mortality within 7 days, the CT model demonstrated increased neutrophil and macrophage recruitment to the lung with 4 h of exposure, but blunted the DC and monocyte recruitment. Adoptive transfer of bulk CD11b⁺ myeloid cells to mice treated with cyclophosphamide alone, but not to CCT mice, protected against IA.

The concept of “trained immunity” was previously reported in a model of systemic candidiasis, whereby a nonlethal exposure subsequently protected mice against a lethal exposure, which was due to the Dectin-1-dependent epigenetic reprogramming of monocytes [18]. This phenomenon has recently been reported with *A. fumigatus* lung exposure; exposing mice deficient in T, B, and natural killer cells to a nonlethal inoculum subsequently protected mice against a lethal exposure [19]. Mechanistically, neutrophils and macrophages were recruited in higher numbers, expressed higher levels of Dectin-1 and CXCR2, and produced early or higher amounts of specific inflammatory cytokines, including IL-1 α , IL-1 β , G-CSF, TNF- α , IL-6, and IL-17A.

Macrophages, Inflammatory Monocytes, Eosinophils, and Natural Killer T Cells

As detailed above, it has long been recognized that neutrophils are essential for defense against IA. However, the innate immune response to *A. fumigatus* in the lung involves the recruitment and/or participation of numerous cell types. Alveolar macrophages have long been recognized as effector cells against *A. fumigatus*. A recent study has shown that this cell type controls the majority of fungal germination [20]. However, some organisms es-

cape the late phagosome and are laterally transferred to other macrophages. Intriguingly, the calcineurin inhibitor FK506 reduces macrophage death and lateral transfer, which results in uncontrolled fungal germination in macrophages. An additional innate cell type recently studied in *A. fumigatus* control is the CCR2+ inflammatory monocyte [20]. These cells are thought to function in transporting fungal antigens from the lung to the draining lymph node, where they prime CD4 T cell responses. Subsequently, a study examining the effects of depleting CCR2+ inflammatory monocytes revealed that these cells are as essential as neutrophils for controlling lung fungal burden [21]. Depleting CCR2+ inflammatory monocytes did not impact chemokine production, neutrophil recruitment after *A. fumigatus* exposure, or neutrophil uptake of conidia. However, neutrophils from mice depleted of CCR2+ inflammatory monocytes were less effective at killing *A. fumigatus* conidia, both in vitro and in vivo, presumably as a result of the impaired production of cytokines (TNF- α) and effector molecules (pentraxin 3 [PTX3]) that enhance neutrophil antifungal activity. In addition, CCR2+ inflammatory monocytes themselves possessed the ability to kill *A. fumigatus*.

The presence of eosinophils has been documented in multiple diseases caused by *A. fumigatus*, including IA [22]. Despite low numbers of eosinophils in the lung after *A. fumigatus* exposure, we have reported that mice deficient in eosinophils demonstrate delayed fungal clearance in the presence of germinating organisms in the lung [22]. Despite blunted proinflammatory cytokine and chemokine levels, there are no defects in innate cell recruitment to the lungs of eosinophil-deficient mice, which suggests that eosinophils themselves must function in *A. fumigatus* killing. Indeed, pure eosinophils inhibit the growth of *A. fumigatus* in vitro, a process that does not require cell contact and can be duplicated by eosinophil cell lysates.

A previous study reported that mice deficient in natural killer T (NKT) cells had delayed clearance of *A. fumigatus* from the lungs [23]. Mechanistically, this report demonstrated that Dectin-1- and MyD88-dependent responses promoted IL-12 production by DCs that drove NKT cells to secrete interferon (IFN)- γ . Building upon this observation, human NKT cells were examined for their response to distinct fungal morphotypes and DCs [24]. It was shown that CD1d+ CD1c+ myeloid DCs were the most potent activator of NKT cells. When live *A. fumigatus* was employed, NKT cells had reduced expression of TNF- α , G-CSF, and RANTES, but not of type 1 or type 2 cytokines.

Membrane-Bound and Soluble Receptors

Toll-Like Receptors

Toll-like receptors (TLRs) are type 1 membrane receptors in a superfamily of 13 transmembrane proteins; 10 are expressed in humans, and 6 of these have been implicated in the recognition of *A. fumigatus*. Many experimental studies have investigated the role of TLRs in IA defense and this will therefore not be discussed in detail here. Regarding immunogenetics, TLR1, TLR6 (coreceptors for TLR2), and TLR4 were the first TLRs recognized as having a genetic susceptibility to IA [25, 26]. Recent studies have uncovered surprising roles for TLR3, which senses RNA, and TLR5, which senses bacterial flagellin, in susceptibility to IA. Mice deficient in TLR3 demonstrate impaired lung fungal clearance which correlates with defective CCR7+ DC migration to the lymph nodes and impaired CD8 T cell responsiveness [27]. Moreover, polymorphisms in TLR3 increase the risk of IA after hematopoietic stem cell transplantation, with these individuals demonstrating impaired IFN-1 and CD8 T cell responses to *A. fumigatus* RNA [27]. Regarding TLR5, although the *A. fumigatus* ligand putatively recognized by TLR5 is not known, in vitro genetic knockdown studies implicated a role for TLR5 in *A. fumigatus* killing [28]. Subsequently, a TLR5 stop single-nucleotide polymorphism (SNP) was identified as an independent risk factor for IA in recipients after allogeneic hematopoietic stem cell transplantation [29]. Finally, although the significance of this observation in IA has yet to be determined, a recent study implicated TLR10, which has no known ligand to date, in chronic cavitary pulmonary aspergillosis [30].

Chitin is a major component of the *A. fumigatus* cell wall, and is usually unmasked when organisms germinate. The PRR for chitin has not been conclusively identified, although TLR2, TLR9, and NOD2 have been implicated as the chitin PRRs in mice, where it drives the production of IL-10 [31]. In human peripheral blood mononuclear cells, chitin purified from the *A. fumigatus* cell wall was recently shown to induce the anti-inflammatory factor IL-1RA, but not the proinflammatory cytokines IL-1 β , TNF- α , IL-6, or IL-8 [32]. In vitro blocking studies revealed that Dectin-1, TLR2, TLR4, the mannose receptor and NOD2 were not involved in the production of IL-1RA. In contrast, chitin-induced IL-1RA production requires the presence of immunoglobulins and Fc γ RII. However, IgG-opsonized chitin is able to synergistically induce IL-1 β production when combined with the ligands for TLR2, TLR4, and NOD2, but not the Dectin-1 ligand.

C-Type Lectin Receptors

The C-type lectin-like receptor Dectin-1 has been extensively studied in host defense against *A. fumigatus*, with deficiency in Dectin-1 in humans and susceptibility to IA having been described. Newer findings regarding Dectin-1 in immune responsiveness to *A. fumigatus* include the observation that Dectin-1 expression is induced in human bronchial epithelial cells in a TLR2-dependent manner, where it promotes the production of inflammatory mediators and antimicrobial peptides [33]. Although NETs have been reported as an antifungal effector mechanism against *A. fumigatus*, a recent study showed that NETs are only formed when *A. fumigatus* is clumped in large aggregates or else in the presence of *A. fumigatus* hyphae [34], thereby contradicting the study on human neutrophils that we mentioned earlier [12]. Intriguingly, Dectin-1 functioned as a negative regulator of NETosis, as NET formation was enhanced in the absence of Dectin-1 and resulted in more tissue damage. Myeloid-derived suppressor cells (MDSCs) are neutrophil-like cells that function to regulate immune responses, often at the level of T cells. A recent study identified the induction of MDSCs in both mice and humans in response to *A. fumigatus* [35]. Subsequently, using cells from humans and mice deficient in Dectin-1 revealed a requirement for Dectin-1 in the development of MDSCs during *A. fumigatus* infection [35].

Dectin-2 is another C-type lectin-like receptor that, unlike Dectin-1, has specificity for fungal mannans. Although not extensively studied in the context of *A. fumigatus* infection, a recent study reported that the cytokines IL-6 and IL-23 induce the expression of the IL-17 receptor IL-17RC on human and mouse neutrophils [36]. Dectin-2 expression is also induced by IL-6 and IL-23 and, when stimulated with an *A. fumigatus* hyphal extract, promotes higher IL-17RC expression, leading to IL-17A-mediated ROS induction and subsequent fungal killing [36]. Likewise, plasmacytoid DCs express Dectin-2 and, upon *A. fumigatus* hyphal recognition, produce inflammatory and type 1 IFN responses as well as form structures that resemble NETs [37].

Pentraxin 3

Early studies revealed a novel, nonredundant role for the long pentraxin, PTX3, in innate defense mediated by alveolar macrophages and neutrophils during IA. The importance of PTX3 in IA defense has now been validated clinically, as a homozygous haplotype in PTX3 was found to be associated with an increased risk of IA in an investigative and validation cohort [38]. This haplotype resulted

in defective expression of PTX3 in neutrophils, leading to impaired phagocytosis and clearance of *A. fumigatus*. As detailed earlier, TLR4 is recognized as an important PRR in IA defense. PTX3 was recently identified as binding to myeloid differentiation protein 2 (MD-2) in vitro and mediating antifungal activity in vivo via TLR4/MD-2-mediated signaling [39]; mechanistically, the engagement of MD-2 by PTX3-opsonized *A. fumigatus* activated a TRIF-mediated IFN- β -dependent signaling pathway. Immunosuppression with drugs such as cyclosporine A or tacrolimus is commonly associated with a higher incidence of IA. A recent study has discovered a mechanism behind this immunosuppression, i.e., targeting the calcineurin-NFAT binding pathways suppresses the expression of PTX3, which leads to increased susceptibility to IA [40].

Ficolins

Ficolins are soluble, secreted PRRs that function to activate the lectin complement pathway. Three different ficolins exist in humans, ficolin-1 (FCN-1/M-ficolin), ficolin-2 (FCN-2/L-ficolin), and ficolin-3 (FCN-3/H-ficolin), while mice only possess an ortholog of human FCN-2, termed ficolin-A. Initial studies demonstrated that although ficolin-A opsonized *A. fumigatus* and had enhanced binding to human lung epithelial cells, ficolin-A-opsonized conidia did not lead to lectin pathway-specific C4 deposition [41]. Ficolin-A opsonized *A. fumigatus* did, however, augment human lung epithelial cell IL-8 production. In contrast, ficolin-A opsonized *A. fumigatus* dampened the production of IL-1 β , IL-6, and TNF- α by human monocyte-derived macrophages and neutrophils, despite enhancing their antifungal activity [42]. Similar results have been reported for L-ficolin [43] and H-ficolin [44], both of which were found to be elevated in bronchial lavage fluid from lung-transplant recipients with documented *A. fumigatus* infection. The role of ficolins in vivo has also recently been reported, whereby mice deficient in ficolin-A experience delayed clearance of *A. fumigatus* from the lungs. However, the mechanism involved is not associated with defective complement activation but rather the impaired induction of inflammatory mediators such as IL-1 β [45].

Effector Cytokines

IL-1 Family

The IL-1 family of cytokines comprises 11 members, 7 with proinflammatory characteristics (IL-1 α , IL-1 β , IL-18, IL-33, IL-36 α , IL-36 β , and IL-36 γ) and 4 with putative

anti-inflammatory characteristics (IL-1Ra, IL-36Ra, IL-37, and IL-38). Surprisingly, the initial study examining IL-1 signaling in IA defense did not identify a protective role [46]. However, this phenotype may be the result of a compensatory increase in IFN- γ production. There has been renewed interest in the role that IL-1 α and IL-1 β play in immune defense, with a recent study challenging the negative role for IL-1R during IA [47]; it demonstrated that IL-1R was essential for survival, with IL-1 α promoting leukocyte recruitment and IL-1 β promoting antifungal activity. This study was different from the previous one regarding organism inoculum, dosing strategy, and the model of IA. A subsequent study examined various inflammasome components in the protection against IA [48]. Here, using the CCT model of immunosuppression-associated IA, mice individually deficient in NLRP3 or AIM2 were not more susceptible than wild-type mice. However, susceptibility was enhanced with combined NLRP3 + AIM2 deficiency (80% mortality by day 15), ASC deficiency (90% mortality by day 15), and dual caspase 1 + caspase 11 deficiency (100% mortality by day 6). DCs from combined NLRP3 + AIM2 deficiency demonstrated impaired IL-1 β and IL-18 production in response to *A. fumigatus* when compared to mice deficient in a single agent. Likewise, similar to the observation above, mice deficient in IL-1 β demonstrated decreased survival (75 vs. 25% mortality in wild-type mice by day 15).

Despite a clear protective role for IL-1R signaling in IA defense, excessive IL-1R signaling may be detrimental. Excessive inflammation observed during IA in chronic granulomatous disease (CGD) is IL-1-dependent and can be ameliorated by IL-1R blockade [49]. Similarly, studies have shown that NLRP3 inflammasome-mediated responses during *A. fumigatus* exposure in murine and human cystic fibrosis (CF) is a result of defective IL-1RA production, which can be minimized by treating with anakinra (recombinant IL-1RA) [50]. Collectively, while IL-1R signaling is essential for the elimination of *A. fumigatus* from the lungs, in specific inflammatory conditions such as CGD and CF, IL-1R signaling rather serves as a therapeutic target for inhibition.

The IL-36 isoforms IL-36 α , IL-36 β , and IL-36 γ are expressed by epithelial cells and monocytes and binds the receptor and coreceptor complex of IL-1Rrp2/IL-1RAcP where they induce proinflammatory responses and antimicrobial factors. The contribution of IL-36 isoforms on T helper responses to *A. fumigatus* has been recently described in humans. Results showed morphologic specific induction of IL-36 α and IL-36 γ , which was dependent on Dectin-1, Syk and TLR4, yet regulated by TLR2 and CR3.

Inhibition of IL-36R resulted in attenuated IL-17 and IFN- γ production in response to *A. fumigatus* [51]. IL-37 is anti-inflammatory cytokine of the IL-1 family that binds IL-18R. Treating *A. fumigatus* infected mice with IL-37 results in inhibition of NLRP3 inflammasome-generated IL-1 β secretion, which was dependent on the IL-1 family decoy receptor TIR-8/SIGIRR [52].

IL-17A, IL-22, and GM-CSF

We and other study groups have reported an important role for IL-17A in lung defense against *A. fumigatus*. Recent studies have now extended this role to the eye, as neutrophils have been identified as a critical source of IL-17A during fungal keratitis [53]. Assessment of JAK/STAT pathway inhibition in this model was shown to have a negative effect on ROS production and fungal killing activity, but also blocked the activity of elastase and gelatinase that is associated with tissue damage [54]. CD4 and $\gamma\delta$ T cells may also produce IL-17A after *A. fumigatus* challenge. Sublethal *A. fumigatus* exposure has also been employed to assess the role of DC subsets in generating Th17, specifically documenting an IRF4-dependent CD11b+CD24+CD64 $^-$ DC population in mucosal tissues with functional specialization to instruct IL-17 responses during fungal infection [55].

We have previously reported a protective role for IL-22 in lung defense against *A. fumigatus* [56], and also that IL-22 contributes to immunopathogenesis in a model of fungal asthma [57]. In the latter study, we reported that CD4 T cells were a significant source of Dectin-1-dependent IL-17A, with IL-22 being produced at much lower levels. However, another study has shown that, in human peripheral blood mononuclear cells, heat-inactivated *A. fumigatus* conidia and hyphae induce high levels of IL-22, primarily in CD4 T cells, although CD8 T cells and NKT cells also serve as an IL-22 cell source [58]. Intriguingly, 10% of the IL-22 produced in these cultures was by an unidentified cell source. Similarly, individuals with CF, who are often colonized with multiple microorganisms, including *A. fumigatus*, demonstrate increased levels of CD4 T cells that produce both IL-17A and IL-22 [59].

GM-CSF has been implicated in the defense against multiple fungal organisms, but its role in *A. fumigatus* lung defense has yet to be characterized. Recently, it was demonstrated that mice deficient in the receptor for GM-CSF were unable to control infection, primarily as a result of the recruitment of inflammatory monocytes being impaired, but not that of neutrophils. However, the defective generation of ROS and also antifungal activity was exhibited by both cell types [60]. GM-CSF treatment was

found to be effective in enhancing neutrophil function and lowering the *A. fumigatus* lung burden, an effect dependent on the generation of ROS.

Processes Affecting Innate Immunity

Autophagy

Autophagy is a lysosomal degradation pathway described as mediating the clearance of multiple pathogens via their engulfment upon escape to the cytosol. Although *A. fumigatus*-associated PRRs expressed on the cell surface are relatively well-characterized, less is known about those expressed in the phagolysosome. However, we have previously reported that innate recognition of ingested resting *A. fumigatus* conidia is mediated by Dectin-1 in acidified phagolysosomes following conidial swelling [61]. This process was recently identified in human monocytes, where Dectin-1-mediated β -glucan recognition of *A. fumigatus* was required for the selective recruitment of LC3 II autophagy proteins [62]. Induction of autophagy was required for organism killing, as the inhibition of ATG5 autophagy proteins impaired phagolysosome fusion. Interestingly, abolishing ROS induction or employing monocytes from CDG patients failed to induce LC3+ phagosome formation following infection with *A. fumigatus*. A similar observation was made when examining monocytes from patients after the administration of corticosteroids. In a follow-up study, it was found that, in addition to β -glucan recognition for LC3 phagolysosome formation, cell wall resolution of melanin is also required [63]. It demonstrated that melanin found in the *A. fumigatus* cell wall, while potentially inducing proinflammatory cytokine production, failed to induce ROS production in human monocytes as a result of specific inhibition of the interaction between the p22phox subunit of flavocytochrome b558 with the NADPH oxidase complex. Importantly, in the aforementioned reports detailing the IA inflammation affiliated with CGD and CF, employing IL-1R blockade restored LC3 phagolysosome formation in the monocytes from CGD patients [49] as well as in the macrophages from mice deficient in CFTR [50].

Hypoxia

A previous study employing 3 different models of IA (cyclophosphamide treatment, corticosteroid treatment, and gp91phox-deficient CGD mice) demonstrated hypoxia in the foci of innate inflammation and *A. fumigatus* organisms [64]. This observation suggests that both the host and *A. fumigatus* must adapt in order to survive in a low-

Table 1. List of reviewed innate immune factors

Name	Gene	References
NADPH oxidase	<i>Cybb</i>	12
Myeloperoxidase	<i>Mpo</i>	12
Lactoferrin	<i>Ltf</i>	12
Protein kinase Cy	<i>Prkcg</i>	14
LC3	<i>Map1lc3a</i>	49, 50, 62, 63
HIF-1 α	<i>Hif1a</i>	66, 67
β -Defensin 1	<i>Defb</i>	69
Dectin-1	<i>Clec7a</i>	14, 19, 33–35, 62
Dectin-2	<i>Clec6a</i>	36, 37
DC-SIGN	<i>Cd209</i>	16
CR3	<i>Itgam/ltgb2</i>	12, 14
TLR3	<i>Tlr3</i>	27
TLR5	<i>Tlr5</i>	28, 29
TLR10	<i>Tlr10</i>	30
PTX3	<i>Ptx3</i>	21, 38, 39, 40
M-ficolin	<i>Fcn1</i>	41, 42
L-ficolin	<i>Fcn2</i>	43
H-ficolin	<i>Fcn3</i>	44
IL-1 α	<i>Il1a</i>	19, 47, 67
IL-1 β	<i>Il1b</i>	19, 32, 45, 47, 48, 67, 69
IL-1RA	<i>Il1rn</i>	49, 50, 67
IL-36 α	<i>Il36a</i>	51
IL-36 γ	<i>Il36g</i>	51
IL-37	<i>Il37</i>	52
IL-17A	<i>Il17a</i>	19, 53–55, 59
IL-22	<i>Il22</i>	58, 59
G-CSF	<i>Csf3</i>	24
GM-CSF	<i>Csf2</i>	60
CXCR2	<i>Cxcr2</i>	19, 67
CCR2	<i>Ccr2</i>	21

oxygen environment. An example of *A. fumigatus* adaptation during hypoxia is the increase in hyphal cell wall thickness and surface-exposed β -glucan content [65]. This results in increased Dectin-1-mediated macrophage and neutrophil inflammatory responses. Hypoxia-inducible factor 1 (HIF-1) α is a host transcription factor that mediates multiple functions during periods of low oxygen. *A. fumigatus* has recently been shown to stabilize HIF-1 α in the lung, a process interrupted by CT [66]. Mice with HIF-1 α deletion in myeloid cells rapidly succumb to IA as a result of impaired neutrophil recruitment to the lungs and enhanced neutrophil apoptosis [66]. A recent study documented that mice deficient in CXCR2 and those immunosuppressed with corticosteroids display defective clearance of *A. fumigatus* from the lungs in the presence of increased hypoxia and increased levels of proinflammatory cytokines, including IL-1 α and IL-1 β [67]. To this end, treatment with the IL-1 blocking agent anakinra limited the de-

Fig. 1. Neutrophils recognize *A. fumigatus* via multiple membrane-expressed (Dectin-1 and CR3) and soluble (PTX3) PRRs, that trigger innate antifungal responses against *A. fumigatus* conidia (lactoferrin) and hyphae (ROS and NETs).

velopment of hypoxia and improved outcome during *A. fumigatus* infection in mice receiving CT, but was not effective in CXCR2-deficient mice [67].

Genome-Wide Association Studies

With the advent of newer technologies, there has been renewed interest in further understanding the genes associated with risk of or susceptibility to IA. In a recent case-controlled study of nearly 800 immunocompromised individuals, 149 of whom were diagnosed with IA, SNPs in IL-4R and IL-8 were associated with increased risk and those in IL-12B and IFN- γ with decreased risk [68]. In fact, patients with the SNP in IFN- γ were found to have better macrophage effector function against *A. fumigatus* and produced more IFN- γ and TNF- α . In another study that focused specifically on recipients of solid-organ transplants, genome-wide association study analysis led to the identification of IL-1 β and β -defensin 1 as essential for limiting fungal colonization and/or infection [69]. Finally, a recent study examining genes in the NF- κ B family found that, despite identifying an SNP in IRF4 that was associated with a 6-fold increased risk of developing IA, this did not reach statistical significance [70].

Conclusion

Table 1 summarizes new findings in the area of innate host defense mechanisms during IA in the last 3–5 years. A better understanding of the genetic immunodeficiency that predisposes to fungal infections has paved the

way for understanding aspects like how innate immune cells (such as neutrophils) recognize (via CR3 and PTX3) and kill *A. fumigatus* conidia (via lactoferrin) and hyphae (via ROS and NETs) (Fig. 1). New roles for TLR3 and TLR5 have been identified and unappreciated roles for new innate cell types, such as inflammatory monocytes and eosinophils, have been discovered. The IL-1 family members IL-1 α and IL-1 β are now recognized as central mediators of *A. fumigatus* clearance, yet they function in a negative role as drivers of unwanted inflammation in diseases such as CGD and CF. Finally, there is new recognition of processes such as autophagy and hypoxia that modulate innate immune responsiveness. With the rise in invasive fungal infections such as IA in a wide range of patients who are both immunocompetent and immunocompromised, it is extremely critical to identify how the mechanisms involved in the innate immune response work together to clear *A. fumigatus* from the lungs. With a better understanding of the PRRs, cytokines, immune cells, and effector mechanisms involved in combating IA, we are provided with more in-depth knowledge of the potential candidates for developing therapies to boost the immune response and prevent or limit infection.

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