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ALMARIN(G) THE INNATE IMMUNE SYSTEM TO INVASIVE FUNGAL INFECTIONS

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Abstract

Fungi encounter numerous stresses in a mammalian host, including the immune system, which they must adapt to in order to grow and cause disease. The host immune system tunes its response to the threat level posed by the invading pathogen. We discuss recent findings on how interleukin (IL)-1 signaling is central to tuning the immune response to the virulence potential of invasive fungi, as well as other pathogens. Moreover, we discuss fungal factors that may drive tissue invasion and destruction that regulate IL-1 cytokine release. Moving forward understanding the mechanisms of fungal adaption to the host, together with understanding how the host innate immune system recognizes invading fungal pathogens will increase our therapeutic options for treatment of invasive fungal infections.

INTRODUCTION

Fungi are ubiquitous in nature and, for the most part, are harmless to the majority of individuals. However, it is estimated that 2 million cases of invasive mycoses are reported worldwide each year [1]. These invasive mycoses occur primarily in immunocompromised patients. Thus, in the absence of an adequate innate host defense, these opportunistic pathogens can infect the host and lead to disease. Furthermore, invasive fungal infections continue to be a rapidly emerging and serious threat because of the growing immunocompromised population and the emergence of drug resistance [1].

Candida spp. and *Aspergillus* spp. are known to cause approximately 30% of all invasive fungal infections [1]. However, the environmental niche filled by *Candida* spp. and *Aspergillus* spp. substantially differs, which could drive evolution of distinct adaptation traits necessary for virulence. *Candida* spp. are found as a normal commensal component of the human skin, gastrointestinal tract and other mucosal surfaces [2], whereas *Aspergillus*

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spp. are saprophytic molds found in the environment on decaying organic material [3]. Hundreds of species exist within the *Candida* and *Aspergillus* genera, however only a handful have been shown to cause invasive mycoses in humans. Even though mortality rates for invasive fungal infections have significantly decreased in the past decade [4], mortality rates from IC and IA are unacceptably high, ranging anywhere from 20–50% due to limited diagnostic tools and lack of effective treatment options [4–9]. Thus, novel therapeutic targets for anti-fungal drugs are desperately needed. Fungal factors driving adaption and growth in the mammalian host offer great potential as novel anti-fungal targets. In addition, tuning the host inflammatory response to confer optimal host resistance is another exciting avenue for limiting invasive fungal infections.

Innate immunity is essential for resistance against *A. fumigatus* and *C. albicans*. Patients with primary immunodeficiencies in the NADPH oxidase complex, STAT3 signaling pathway, CARD9 signaling pathway, IL-17 immunity, leukocyte adhesion deficiencies, and those with severe congenital neutropenia have been shown to be predisposed to developing invasive fungal infections (reviewed in [10] and [11]). Moreover, polymorphisms in numerous innate immune sensing and signaling pathways alter the susceptibility of transplant patients to developing invasive fungal disease (reviewed in [11], [12] and [13]). In this review, we discuss the importance of interleukin-1 (IL-1) in tuning the inflammatory response in the context of invasive fungal disease. Moreover, we highlight the potential importance of this model broadly across the spectrum of infectious diseases. We highlight recent data which demonstrate that the mammalian innate immune system responds in a regulated manner that is tuned to the level of growth, virulence, and pathology induced by the fungal pathogen.

ALARMINS/DAMAGE-ASSOCIATED MOLECULAR PATTERNS (DAMPs) VERSUS MICROBIAL-ASSOCIATED MOLECULAR PATTERNS (PAMPs)

The innate immune system provides an essential early response to microbial infection. Initial sensing of microbes has been well established to be mediated by a series of germline-encoded host pattern-recognition receptor (PRR) families, including Toll-like receptors (TLRs), C-type lectin receptors (CLRs), Nod-like receptors (NLRs), and RIG-I-like receptors (RLRs), which can recognize conserved microbial structures termed microbial-associated molecular patterns (MAMPs). Examples of MAMPs of particular relevance to fungal pathogens include β-1,3-glucan, chitin, mannans, mannoproteins, and unmethylated DNA. However, all fungi whether pathogenic to the host or not will express these MAMPs. To address the conceptual problem of pathogen versus commensal organism, Vance and colleagues have proposed that it is not just the MAMP that is critical for immune cell activation, but also its location within the host and/or cell, which they termed the “patterns of pathogenesis” [14]. These early pathogenic signatures will instigate the inflammatory response following fungal exposure. During the course of invasive fungal infections, fungal growth and invasion into the body, together with the host immune response will cause significant tissue damage, extracellular matrix destruction, and cell death at the site of infection. Tissue destruction and cell death during infection is highly inflammatory due to the release of damage-associated molecular patterns (DAMPs) or alarmins from the dying

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host cells [15, 16]. These alarmins include both non-protein materials, such as ATP and uric acid, as well as proteins, which include IL-1α, IL-33, S100 proteins, and HMGB1. Thus, during invasive fungal infections initial inflammation will be regulated by PRRs, but if that response is insufficient to prevent invasive growth, alarmin release due to tissue destruction and host cell death will amplify the magnitude of the inflammatory response to attempt to regain control (Figure 1).

BASICS OF THE IL-1 CYTOKINES IN INFLAMMATION

The IL-1 gene cluster codes for the cytokines IL-1α and IL-1β, as well as the IL-1 receptor antagonist (IL-1ra), all three of which can bind to the IL-1 receptor, type I (IL-1RI) [17]. While IL-1α and IL-1β are pro-inflammatory cytokines, IL-1ra competitively binds the IL-1RI to dampen the immune response [17]. Although IL-1α and IL-1β belong to the same cytokine family, they rely on different proteases and cell death pathways for their secretion. IL-1β is produced as an inactive precursor termed pro-IL-1β that must first be transcriptionally upregulated [18]. For secretion, Pro-IL-1β must then be cleaved by a caspase-1 or caspase-8 containing inflammasome, which is formed following NLR activation [18]. Ultimately, IL-1β is released by pryoptotic cell death, which is mediated by gasdermin D [19–21]. Alternatively, pro-IL-1β can be cleaved by neutrophil-derived proteases, such as elastase, cathepsin G, proteinase 3, and granzyme A, which might be important in neutrophil-rich inflammatory settings, such as those observed during later stages of invasive fungal infections [22]. Unlike IL-1β, pro-IL-1α is constitutively expressed in cells. IL-1α can be released either as pro-IL-1α or mature IL-1α after calpain cleavage, but in either form it can bind to IL-1RI to initiate signaling [17, 23]. IL-1α is typically released during highly pathological situations, including necrotic and necroptotic cell death [17, 24]. Biologically, IL-1α and IL-1β can have different inflammatory activities in certain inflammatory settings [25–27], including during fungal infections [28, 29]. This was most pointedly demonstrated by the work of Rider *et al* which demonstrated that IL-1α released by cells undergoing necrotic cell death is necessary for neutrophil accumulation to initiate a sterile inflammatory response, while later IL-1β release promotes macrophage accumulation [26]. However, the distinct roles of IL-1α and IL-1β during infections is only beginning to be appreciated.

ROLE OF IL-1α and IL-1β DURING INVASIVE FUNGAL INFECTIONS

Candida albicans

IL-1 signaling is essential for immunity against candidiasis, as immune competent *Il1r1*-deficient and *Il1a/Il1b*-deficient mice are both highly susceptible to *C. albicans* infection [28, 30]. Moreover, treatment of neutropenic mice with either recombinant IL-1α or IL-1β was able to partially protect those mice [31]. Interestingly, IL-1α and IL-1β serve non-redundant roles in the immune response against systemic candidiasis (Table 1) [28]. Specifically, IL-1β is needed for optimal neutrophil recruitment, whereas IL-1α was needed to enhance anti-fungal activity of neutrophils [28].

When activated by *C. albicans*, pro-IL-1β transcription is regulated through a Syk-Card9 pathway and IL-1β release is dependent on the NLRP3 and NLRC4 inflammasomes [32–

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35]. Mice lacking either inflammasome are highly susceptible to disseminated candidiasis [32–35]. Initially, NLRP3 activation in macrophages was thought to be dependent on the morphological switch of *C. albicans* from yeast to filamentous form, but is independent of the actual filament because both pseudohyphae and hyphae are capable of inducing IL-1β secretion [34]. More recent data from screening multiple *C. albicans* genetic mutant libraries demonstrate that filamentation is not sufficient for inflammasome activation to drive IL-1β release and pyroptosis [36–38]. These screens were able to identify both mutants that could filament, but not cause pyroptosis and mutants that were unable to filament, but could still drive pyroptosis. Interestingly, heat-killed *C. albicans* could not activate macrophage pyroptosis, but if the *C. albicans* that was previously phagocytized by macrophages for ~1h were heat-killed, they could induce robust pyroptosis [38]. Taken together, these data led Cowen and colleagues to postulate that *C. albicans* remodels its cell surface in response to macrophage phagocytosis. In an elegant experiment, Cowen and colleagues treated previously phagocytized *C. albicans* with the Endo H glycosidase prior to heat killing and observed a complete loss of pyroptosis, suggesting highly mannosylated surface proteins were required to induce macrophage pyroptosis [38]. In addition to this early pyroptotic cell death, *C. albicans* can also induce macrophage cell death in a pyroptosis-independent manner at later times that is highly dependent on the presence of fungal filaments, which likely are mechanically piercing the cell [39]. Whether these two phases of macrophage cell death result in differential activation and release of IL-1α and IL-1β has not been explored.

These prior studies focused largely on the interaction of *C. albicans* with macrophages. *C. albicans* is a normal member of the mucosal microbiota and, thus, would have to invade the epithelium to cause diseases. Thus, the transition of *Candida* spp. from the yeast form into the filamentous form can be thought of as an important virulence determinant of the pathogen, enabling it to invade through the epithelium and grow within the host [40, 41], but likely would also cause significant tissue pathology due to destruction of the epithelium barrier and alter the inflammatory environment. Interestingly, in an *in vitro* oral candidiasis model in which human epithelial cells were infected with *Candida* spp. or *C. albicans* mutants that either can or cannot form hyphae, IL-1α expression was increased during the infections with *Candida* spp. that are able to form hyphae [42–44]. However, a thorough dissection of this oral epithelium model with the *C. albicans* mutant libraries, as was done in the IL-1β and pyroptosis studies discussed above, has not been undertaken to examine how IL-1α release is controlled. Taken together, these studies support our model that fungal morphotype can influence the release of alarmins, specifically IL-1α, by creating a high-threat environment in which a quick response by neighboring cells is critical, and prevention of collateral damage is not as important as clearing the threat.

Aspergillus fumigatus

IL-1 signaling is essential to control *A. fumigatus* growth, but controversy still exists on the relative importance of IL-1α and IL-1β following *A. fumigatus* challenge (Table 1) [29, 45–48]. Our group has shown that IL-1α was required for neutrophil recruitment after pulmonary challenge with *A. fumigatus*, while the inflammasome and IL-1β were necessary for optimal fungicidal activity of macrophages [29]. However, others have shown the inflammasome and IL-1β are necessary for neutrophil recruitment following *A. fumigatus*

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challenge [47, 49]. Both the NLRP3 and AIM2 inflammasomes work in concert to generate an optimal anti-*Aspergillus* response [47, 49]. At least *in vitro*, activation of the NLRP3 inflammasome by *A. fumigatus* is greatest by hyphal fragments [50]. However, compared to *Candida*, there is a lack of data concerning how distinct fungal morphotypes of *Aspergillus* spp. may relate to overall pathology and inflammation. It is important to note that in the above mentioned studies in which discrepancies concerning IL-1 dependency arise, different *A. fumigatus* strains and morphotypes were used [29, 47, 49]. Interestingly, it is rapidly emerging that different strains of *A. fumigatus* can induce both dramatically different inflammatory responses [51, 52] and differing levels of virulence *in vivo* [52–54]. Interestingly, Rizzetto *et al* showed that the CEA10 isolate of *A. fumigatus* induced the greatest inflammation, which corresponded with its increased growth in immune competent mice [52]. This raises the possibility that different strains of *A. fumigatus* might undergo differential growth in the respiratory tract which will induce varying degrees of tissue pathology, resulting in the differential release of IL-1α and IL-1β, or generally alter the inflammatory response.

Invasive aspergillosis is a spectrum of diseases in the clinic. In neutropenic hosts, pathology is driven by excessive fungal growth [55], whereas in hosts immunosuppressed with steroids, patients with chronic granulomatous disease (CGD), or patients with cystic fibrosis, pathology is driven by an inflammatory response that appears out of proportion to hyphal growth in diseased tissues [55–57], which might alter the type of immunotherapeutic intervention one might consider (Figure 2). With this prior observation in mind, van de Veerdonk and colleagues explored whether immunotherapeutic intervention with anakinra (recombinant hIL-1Ra) to decrease inflammation could ameliorate IA in the murine model of X-linked CGD. Interestingly, blockade of IL-1 signaling using anakinra (recombinant hIL-1Ra) in the murine model of CGD significantly blunted IA severity [58]. Similarly, anakinra (recombinant hIL-1Ra) treatment of *Cftr*$^{-/-}$ mice, which are more susceptible to IA [59], also resulted in an amelioration of *Aspergillus*-induced mortality that was associated with decreased fungal growth and inflammation [57]. In both cases, the authors have not specifically addressed whether excessive IL-1α and/or IL-1β signaling was responsible for disease caused in these IA models. Interestingly, cells from CGD patients are known to secrete significantly greater amounts of IL-1α and IL-1β in response to inflammatory stimuli [58, 60]. Considering the earlier finding that neutropenic mice infected with *C. albicans* and subsequently treated with either recombinant IL-1α or IL-1β were partially protected from disease [31], it will be intriguing to assess whether similar cytokine therapies might enhance protective immune responses in neutropenic models of IA. Overall, it is possible that *Aspergillus* spp. might induce varying degrees of tissue pathology in each of the clinically relevant models of IA, resulting in the differential release of IL-1α and IL-1β and, therefore, may require unique therapeutic interventions (Figure 2).

FUNGAL TARGETS THAT CONTRIBUTE TO TUNING HOST PATHOLOGY, CELL DEATH, AND IL-1 IMMUNITY

During invasive fungal infections, the initial fungal morphotype that establishes an infection within the mammalian host likely is not the most inflammatory fungal morphotype. This is

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especially true with *A. fumigatus* as the conidia have a hydrophobin layer that is immunologically inert and is responsible for hiding the underlying carbohydrate cell wall from the immune system [61]. Upon seeding the environmentally exposed mucosa of the mammalian host, both *A. fumigatus* and *C. albicans* encounter the mucosal epithelium which provides a barrier against growth inside the host. It is recognized that invasion of the host is typically mediated by a transition to filamentous growth, which also corresponds with immunogenicity of the fungi. Thus, understanding both the molecular switch(s) for filamentous growth and its resultant changes in fungal biology will be essential for limiting tissue pathology and inflammation associated with invasive growth.

During the transition to filamentous growth, rearrangement of cell wall components is critical for immune activation through CLRs and NLRP3 inflammasome activation [34, 38, 50]. β-1,3-glucans have been shown to induce both pro-IL1β expression, as well as induce its maturation by the NLRP3 inflammasome [62]. More recently, highly mannosylated cell wall proteins in *C. albicans* were determined to induce macrophage pyroptosis through inflammasome activation [38]. Specific to *A. fumigatus*, a novel cell wall carbohydrate, galactosaminogalactan (GAG), has emerged as an important virulence determinant that can also regulate IL-1 signaling and cell death [58, 63–65]. GAG is not expressed by resting conidia, but is highly expressed on germtubes and hyphae [65]. GAG also exists in both acetylated and deacetylated forms in the cell wall [66]. GAG has several immunomodulatory functions. First, it masks β-1,3-glucans from immune recognition in the hyphal cell wall [63, 67]. Second, GAG can induce IL-1ra, which inhibits IL-1 signaling and ultimately impairs neutrophil recruitment to the site of infection [58], though others have not observed this [64]. The reason for these discrepant findings remains to be elucidated, but the experimental design differed significantly between those studies. Specifically, Latgé and colleagues have exogenously treated mice with urea-soluble GAG [58, 65], while Sheppard and colleagues expressed the *A. fumigatus* Uge3 enzyme in *Aspergillus nidulans*, which typically cannot make GAG [64]. Third, GAG contributes to resistance against NADPH-oxidase induced neutrophil extracellular traps (NETs) [64]. Finally, GAG induces cell death in both epithelial cells and peripheral blood neutrophils, but the mechanism(s) of cell death induced remains inconclusive [63, 65]. GAG is critical for *A. fumigatus* adherence to epithelial cells, which is likely important for its induction of cell death [63, 67]. Moreover, it has recently been shown that the deacetylated form of GAG is necessary for adhesion and virulence [66]. Thus, a greater understanding of fungal cell wall carbohydrate and glycoprotein expression, biosynthesis, and exposure on distinct fungal morphotypes is necessary for understanding cell adhesion, invasive growth, cell death, and production of the IL-1 cytokine family during invasive fungal infections.

The founding infectious fungi must also rapidly adapt to the environmental and nutritional stresses of its mammalian niche to grow. Among all eukaryotic organisms, cAMP and MAP kinase pathways are conserved and required for the transcriptional regulation of cell growth and differentiation processes in response to specific extracellular stimuli. In both *A. fumigatus* and *C. albicans* it is well established that MAP kinase and cAMP signaling are necessary for full virulence [68]. Adaptation to neutral-alkaline pH environments that are encountered within the mammalian host is one environmental stress invasive fungi most overcome, and this has been shown to occur through Rim101/PacC signaling. The Rim101

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mutant of *C. albicans* was unable to undergo the morphological change from yeast to hyphal growth when grown on basic media, and has been shown to have defects in cellular and tissue invasion *in vitro* and *in vivo* [69–72]. In an *in vitro* oral epithelium model of *C. albicans* infection, a Rim101 mutant was unable to induce as much cell damage, which corresponded with decreased expression of IL-1α, TNFα, and IL-8 compared to its parental or complemented strains [72]. Interestingly, the PacC mutant of *A. fumigatus* did not have a defect in hyphal growth, but PacC-mediated signaling was crucial for *in vitro* epithelial cell destruction and tissue invasion in a neutropenic murine model of IA [73]. *In vitro*, epithelial cell destruction by *A. fumigatus* occurred in a biphashic manner in which the early cell death (<16h) was dependent on direct fungal-epithelial cell contact, whereas later cell death (>16h) was partially dependent on a secreted protease that is antipain sensitive [73]. This later finding with the PacC-null mutant of *A. fumigatus* demonstrates that secreted factors that are expressed during hyphal growth are likely critical in host pathology and cell death, which will likely skew the inflammatory environment, including the IL-1 cytokines. In support of this, deletion of the transcription factor PrtT, which controls the expression of multiple *Aspergillus* proteases, resulted in an *A. fumigatus* mutant strain that was less cytotoxic to lung epithelial cells in *in vitro* assays [74]. While our understanding of the role of proteases in IA is limited, much more is known about the role of *Aspergillus* proteases in chronic airway disease and inflammation. Fungal proteases are sufficient to induce inflammation and allergic airway diseases [75]. A protease isolated from *Aspergillus melleus* was sufficient to induce allergic airway diseases through a unique TLR4-dependent inflammatory pathway that included increased expression of both IL-1α and IL-1β [75]. During invasive *C. albicans* infections, secreted aspartyl proteinases (SAPs) have been identified and are associated with hyphal formation, tissue damage, and virulence [76]. Moreover, internalized Sap2 and Sap6 could mediate NLRP3 inflammasome activation in human monocytes leading to IL-1β release [77]. Sap2 was also sufficient to drive IL-1β-dependent inflammation in a vaginitis model and contributed to the inflammation observed during *C. albicans*-induced vaginitis [78]. Thus, many more studies examining the role of extracellular proteases, as well as other secreted factors, are needed to understand invasive fungal disease, tissue pathology, cell death, and inflammation.

IS THERE A UNIVERSAL IMPORTANCE OF SENSING PATHOLOGICAL POTENTIAL OF PATHOGENS: LESSONS FROM BACTERIA AND VIRUSES?

Bacteria

This idea of an escalating immune response based on the level of threat posed to the immune system has also been demonstrated in the context of bacterial infections. Both *Pseudomonas aeruginosa* and *Staphylococcus aureus* can cause highly inflammatory pulmonary disease resulting in substantial damage and destruction of the lung tissue. Highly virulent strains of *P. aeruginosa* possess a cytotoxin named ExoU that is known to cause epithelial damage and necrotic cell death [79, 80]. Infection with *exoU*-encoding strains results in neutrophil recruitment that is dependent on IL-1α, but when the *exoU* gene was deleted, neutrophil recruitment switched to be dependent on IL-1β signaling [81]. Similarly, during infection with the highly virulent USA300 strain of *S. aureus*, it has been shown that bacterial toxins induce necroptosis of host cells, which largely contributes to the highly inflammatory

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pathology seen during *S. aureus*-induced pneumonia [82]. Thus, it appears that highly pathological bacterial strains that encode cytotoxic exotoxins can drive rapid necrotic host cell death which correlated with IL-1α release from the dying cells.

Viruses

New viral virions can bud from infected cells either in a cytopathic (lytic) or non-cytopathic manner. During cutaneous infection with cytopathic herpes simplex virus-1 (HSV-1), IL-1α is essential for leukocyte recruitment and restriction of viral dissemination [83]. Likewise, in a model of disseminated adenovirus infection, cooperation between IL-1α signaling and the complement system were shown to induce neutrophil recruitment and subsequent clearance of virus-containing macrophages [84]. In this particular system, the presence of a mutated viral protease p23, in which the virus cannot induce endosomal rupture, IL-1α levels were significantly lower resulting in an overall reduced inflammatory response [85]. Finally, in pigs experimentally infected with the cytopathic porcine reproductive and respiratory syndrome virus (PPRSV), the degree of lung pathology observed 7 days after challenge correlated with IL-1α expression [86]. This suggests that host cell damage and IL-1α release is a central part of activating the antiviral immune response to viruses that have cytopathic effects.

CONCLUDING REMARKS

As highlighted in this review, IL-1 signaling is a critical signaling hub in host defense against infectious disease, spanning fungi, bacteria and viruses. In the context of innate anti-fungal immunity, we propose a model in which the immune system responds in an escalating manner, based on the threat posed by the specific invading pathogen in accordance to its virulence and pathological potential (Figure 1). The first level of protection lies in the physical barrier functions of the epithelial layer, which prevents the pathogen from gaining access to the body. Additionally, tissue sentinel immune cells patrol the epithelial barrier and can phagocytose and kill microbes with limited virulence potential, in an inflammasome and IL-1β-dependent manner. However, when a pathogen takes on a highly virulent phenotype, in which it can rapidly adapt to the new environment, it can cause epithelial cell destruction, tissue damage, and cell death. In this latter scenario, IL-1α will be released from host cells to act as a rapid danger or alarmin signal to surrounding cells. In this “last resort” scenario, limiting the amount of tissue damage caused by the immune system is less important than clearing the threat posed by the pathogen to the host. Overall, by understanding all the factors that contribute to increased virulence and high levels of damage to the host, we can discover novel therapeutic strategies to combat a number of infectious diseases.

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HIGHLIGHTS

- IL-1α and IL-1β have non-redundant role immunity against invasive fungal infections
- Pathogens that induce greater tissue damage tend to drive IL-1α release
- Fungal effectors driving tissue pathology and damage largely remain unidentified
- Manipulation of the host immune response may be a potential avenue to treat invasive fungal infections

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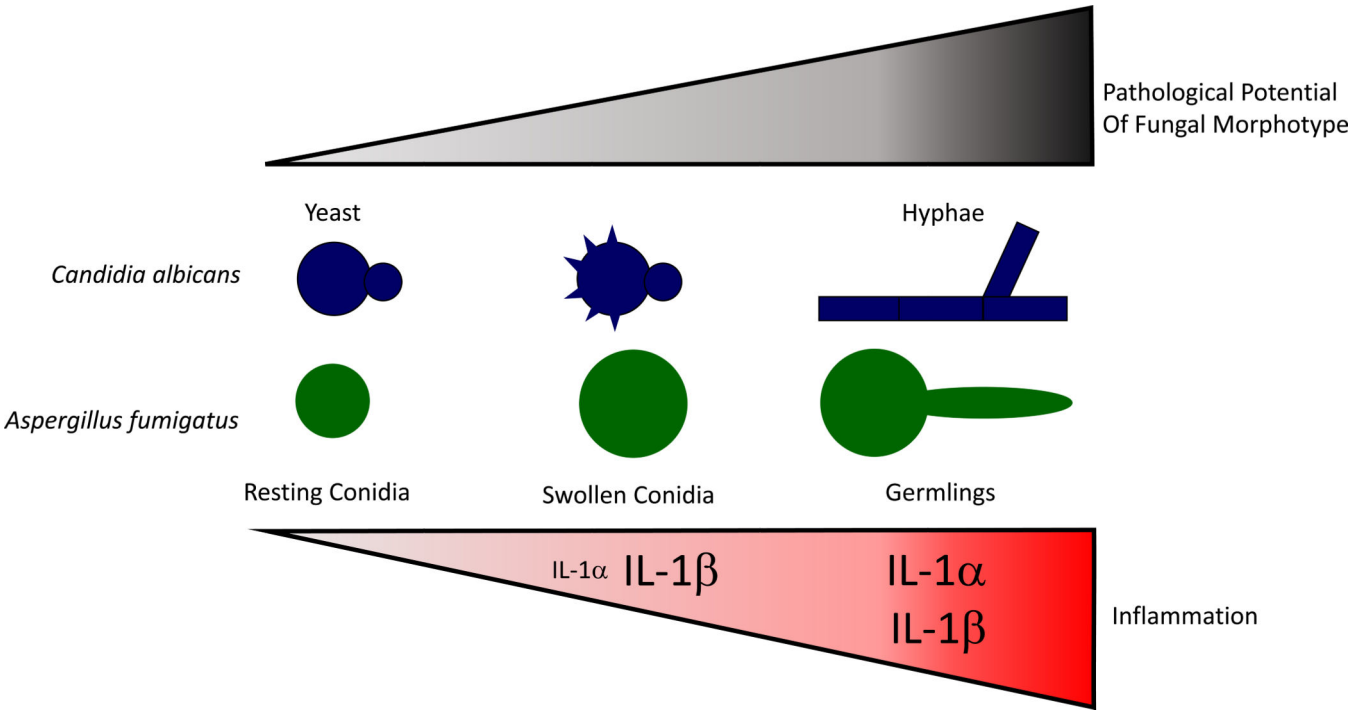

Figure 1. Escalating IL-1 inflammatory response regulates mammalian resistance to invasive, pathological fungal infection

In homeostatic conditions when the barrier to mucosal surfaces is intact, *C. albicans* and *A. fumigatus* can be found in a resting state on mucosal surfaces and in the airways, respectively. Once this primary immune barrier is breached, fungi can access nutrients necessary for growth. In the case of *C. albicans*, rearrangement of the cell wall occurs during phagocytosis, while in the case of *Aspergillus* spp. the cell wall architecture is changed upon conidial swelling. In either case, fungal MAMPs are revealed to the immune system resulting in the activation of an inflammasome-dependent immune responses to clear the fungal threat. If the fungi continue to grow, forming invasive hyphae and expressing hyphal-specific effectors, including GAG, secondary metabolites, and proteases, robust tissue pathology can result. This increased pathology drives elevated levels of pro-inflammatory cytokines and alarmin release, such as IL-1α, which intensify the innate immune response in hope of clearing the infection. Font size for each cytokine is indicative of their relative abundance.

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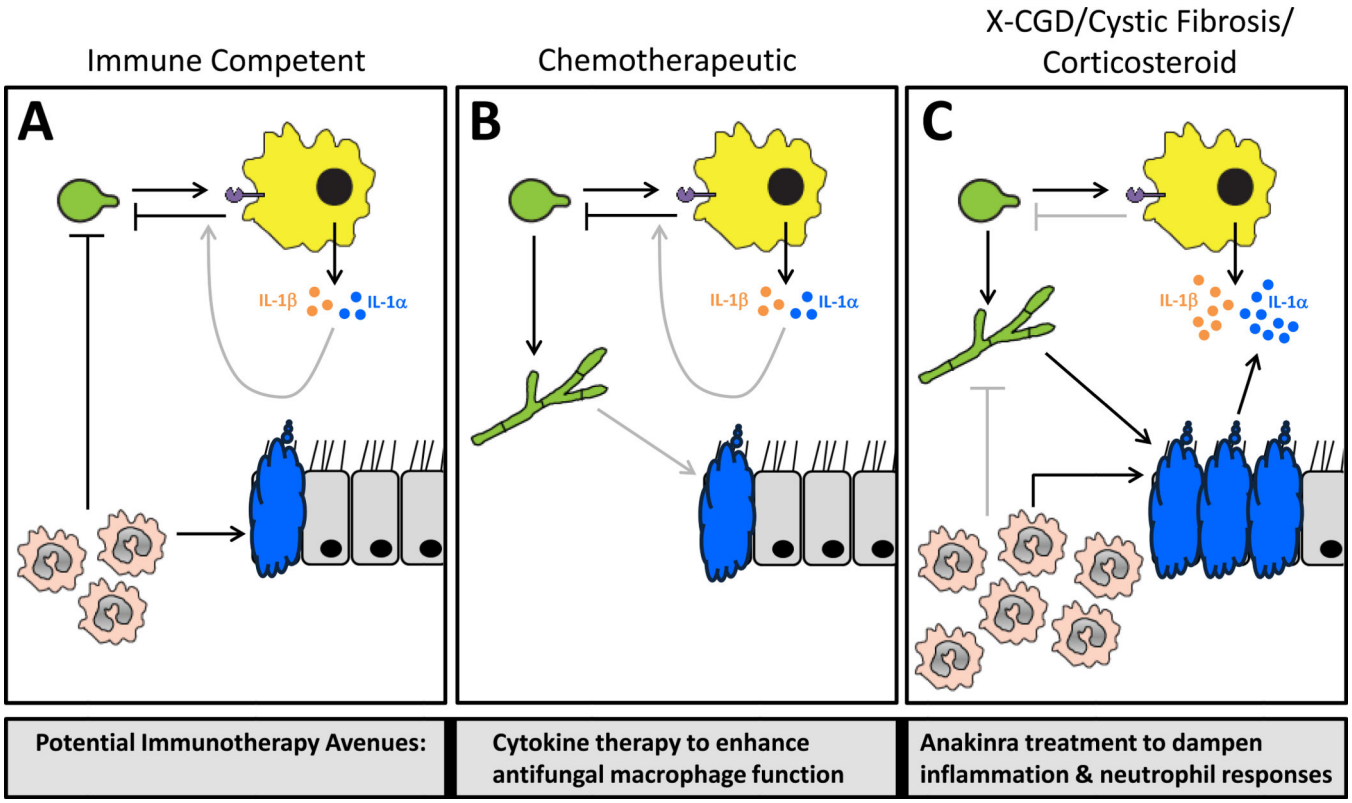

Figure 2. Differential tissue damage and IL-1 mediated inflammation in clinical models of IA will necessitate different immunotherapeutic interventions

(A) In immune competent hosts, germinating *Aspergillus* conidia are rapidly recognized by tissue-resident leukocytes, which results in the release of IL-1α and IL-1β and the recruitment of neutrophils to the site of infection. Neutrophils then exert their powerful antifungal effects to limit *Aspergillus* growth. Additionally, IL-1α/β may work to enhance the antifungal functions of macrophages. (B) In neutropenic hosts, typically due to cyclophosphamide treatment, the lack of neutrophils allows extensive fungal growth. In these hosts, macrophages will still exert their antifungal function, but this is obviously not sufficient to prevent IA. In this population it would likely be advantageous to treat with inflammatory cytokines to further enhance monocyte/macrophage recruitment to the respiratory tract and/or their antifungal effector functions. (C) In CGD patients, cystic fibrosis patients, and individuals treated with high-doses of corticosteroids there is significant inflammation, neutrophil recruitment, and tissue damage associated with IA. However, due likely to impaired antifungal activity of the monocytes/macrophages and neutrophils in these populations, the host is unable to effectively clear the *Aspergillus* leading to continued inflammation. In these populations it has been shown to be advantageous to treat with anti-inflammatory compounds, such as anakinra, in order to limit inflammation, neutrophil recruitment, and tissue damage to the respiratory tract.

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TABLE 1

Functions of IL-1α and IL-1β during *C. albicans* and *A. fumigatus* infection.

	IL-1α	**IL-1β**
	Recombinant IL-1α increases survival in a neutropenic mouse model of invasive candidiasis [31]	Recombinant IL-1β increases survival in a neutropenic mouse model of invasive candidiasis [31]
C. albicans	Endogenous IL-1α needed for optimal antifungal activity of neutrophils against pseudohyphae, and Th1 response [28]	Endogenous IL-1β needed for recruitment of neutrophils, superoxide generation, and Th1 response [28]
		NLRP3 and NLRC4-dependent IL-1β production [32–35]
	IL-1α needed for neutrophil recruitment [29]	IL-1β needed for optimal antifungal activity of macrophages [29]
A. fumigatus		NLRP3- and AIM2- dependent IL-1β production [47, 49, 50]
		NLRP3-dependent neutrophil recruitment [47, 49]