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Review article

# New advances in invasive aspergillosis immunobiology leading the way towards personalized therapeutic approaches

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ABSTRACT

Invasive aspergillosis (IA) remains a devastating disease in immune compromised patients despite significant advances in our understanding of fungal virulence and host defense mechanisms. In this review, we summarize important research advances in the fight against IA with particular focus on early events in the interactions between *Aspergillus fumigatus* and the host that occur in the respiratory tract. Advances in understanding mechanisms of immune effector cell recruitment, antifungal effector mechanisms, and how the dynamic host-fungal interaction alters the local microenvironment to effect outcomes are highlighted. These advances illustrate exciting new therapeutic opportunities, but also emphasize the importance of understanding each unique fungus-host interaction for improving patient outcomes.



**Contents**



## 1. Introduction

Humans breathe 10–15 $m^3$ of air daily, a volume that typically contains several hundred to several thousand airborne *Aspergillus* conidia [109]. For the most part, this lifelong encounter results in asymptomatic fungal clearance, facilitated in part by mucociliary clearance mechanisms. However, owing to their small size (i.e.

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2–3 μm in diameter), inhaled conidia can reach terminal airways where they activate the respiratory innate immune system via soluble and membrane-bound receptors. Critical immune effector cells, including neutrophils, inflammatory monocytes, and macrophages, must be recruited and activated to thwart fungal invasion.

Inhalation of *Aspergillus* conidia results in their deposition into a new microenvironment. In immune competent murine models of invasive aspergillosis (IA), germination of *Aspergillus* conidia in the airways is rarely observed, although there appears to be some strain dependency that remains to be fully appreciated [92]. However, the large inocula typically used to establish a bronchopneumonia in these murine models results in significant inflammation in the lower airways of the lung. While large inocula of fungal conidia are plausible under specific exposure conditions (e.g., during mulching/gardening), it is unclear whether inocula capable of inducing robust airway inflammation in mice are responsible for disease development in susceptible patient populations. Yet, even a low number of inhaled fungal conidia likely induce localized microenvironment changes that can impact subsequent host immune responses in immune compromised patients. How this microenvironment impacts IA outcomes has not been defined and many open questions remain. For example, questions such as how the microenvironment alters fungal pathogen associated molecular pattern exposure (PAMP) and virulence and how the microenvironment alters immune signaling and antifungal activity of phagocytes such as macrophages, monocytes, and neutrophils remain under appreciated.

Neutrophils are one of the key inflammatory cells that mediate resistance against infection with *Aspergillus fumigatus.* This is highlighted by the observation that patients who become neutropenic after chemotherapy are at a higher risk for developing IA [33]. Animal models clearly demonstrate that timely neutrophil recruitment to the respiratory tract following *A. fumigatus* exposure is critical for resistance to invasive disease and control of fungal growth [9,77]. Moreover, appropriate neutrophil activation and antifungal activity are necessary for resistance to invasive *A. fumigatus* infection as both patients with chronic granulomatous disease (CGD) and mice that lack NADPH oxidase subunits are highly susceptible to infection [83,90]. However, recent epidemiological studies suggest that the incidence of IA is also increasing in non-neutropenic patients [103]. Interestingly, IA in chronic granulomatous disease or following corticosteroid immunosuppression is associated with excessive accumulation of functionally impaired neutrophils that contribute to tissue damage [4,25]. Therefore, precise regulation and calibration of pulmonary inflammation and leukocyte recruitment may ameliorate disease in these contexts. Surprisingly, our understanding of neutrophil recruitment and activation in response to *A. fumigatus* challenge has been slow to emerge. The goal of this mini-review is to highlight new advances in the last 3 years regarding our understanding of how the immune system prevents host damage from *A. fumigatus* challenge. We focus on recognition of the fungus as it enters the airway and mechanisms of immune cell recruitment, activation, and antifungal effector activity. Fungal components that effect these interactions with the host are discussed, and we discuss emerging awareness of how changes in the tissue microenvironment, such as oxygen and nutrient levels, can alter the outcome of the host-pathogen interaction.

A last point of emphasis is that emerging studies strongly suggest, perhaps not surprisingly, that the immune response to a given strain of *A. fumigatus* is not stereotypical. Thus, understanding this strain-specific variation represents a major gap in knowledge that is particularly relevant to design immune-enhancing or immune-modulating strategies (a goal of personalized medicine) for patients afflicted by this menacing mold. Consequently, a dynamic infection microenvironment, strain heterogeneity, patient genetic variability, diverse underlying disease conditions, and a limited antifungal arsenal all contribute to the complex and significant challenge inherent in improving IA outcomes.

## 2. *Aspergillus* recognition and innate immune activation

### 2.1. Pentraxin-3

Pentraxin-3 (*Ptx3*), a soluble collectin and acute phase reactant, binds to conidia and this interaction can be inhibited by soluble galactomannan *in vitro* [31]. Pentraxin-3 regulates complement interactions with fungal conidia and promotes neutrophil conidial uptake by a complement-, CD18/CD11b (a.k.a complement receptor 3/Mac-1)-, and FcγRII-dependent mechanisms [80]. Pentraxin-3 binds the Toll-like receptor (TLR) 4 accessory protein myeloid differentiation protein 2 (MD-2) can modulate lung inflammation via the TLR4/MD-2/CD14 signal transducer TIR domain-containing adapter inducing interferon-β (TRIF) to promote fungal clearance [11]. Consistent with this model, Ptx3$^{-/-}$ and MD-2$^{-/-}$ mice are susceptible to conidial challenge compared to control mice [11,31]. In humans, receipt of a donor graft with a specific pentraxin-3 gene variant increases the likelihood of developing IA during hematopoietic cell transplantation [23].

### 2.2. Fungal interactions with lung-resident leukocytes through CLRs

Alveolar macrophages can rapidly phagocytose conidia within airways and this engulfment process can be blocked *in vitro* by antibodies directed against dendritic cell-specific intercellular adhesion molecule 3-grabbing nonintegrin (DC-SIGN/CD209) [99]. Resting conidia express FleA, a protein that binds to fucosylated molecules found in mucinous secretions [61]. Conidial phagocytosis by macrophages can be disrupted by soluble fucose moieties and Δ*fleA* conidia are not engulfed at a rate comparable to wild-type conidia. These data support the notion that FleA mediates conidial interactions with phagocytic cells, though the macrophage receptor(s) responsible for these interactions have not been functionally characterized. Interestingly, the Δ*fleA* strain is hypervirulent compared to its parental strain, which correlates with significantly greater germination and growth in the airways. Collectively, these data suggest that host recognition of *A. fumigatus* FleA contributes to fungal clearance and control *in vivo* [61].

Although resident alveolar macrophages and other phagocytes rapidly phagocytose resting conidia, engulfed conidia do not trigger robust inflammatory responses prior to conidial swelling, the first step in germination. The conidial surface consists of a layer of hydrophobins that are encoded by the *rodA* and *rodB* genes in *A. fumigatus* and that conceal an underlying layer of fungal β-(1,3) glucan [1]. Conidial swelling coincides with the obligate exposure of particulate β-(1,3) glucan [48] and other cell wall polysaccharides. These polysaccharides are actively recognized by the host to initiate the antifungal immune response.

The C-type lectin receptor Dectin-1 (*Clec7a*) binds to β-glucans on germinating conidia [34,48,102]. Dectin-1 binding to β-glucan extrudes the regulatory phosphatases CD45 and CD148 from the vicinity of receptor-particulate β-glucan complexes [35] (Fig. 1). These events induce Src-dependent phosphorylation of the ITAM-like motif found in the intracellular domain of Dectin-1 and facilitate the recruitment of the SHP-2 phosphatase [26]. Spleen tyrosine kinase (Syk) docks to this scaffold and transduces signals via protein kinase C (PKC)-δ [104] to CARD9, which complexes with B cell CLL/lymphoma 10 (BCL10) and Mucosa-associated lymphoid tissue lymphoma translocation protein 1 (MALT1) [43] in order to activate NF-κB-dependent cytokine production (i.e., IL-6, IL-12, IL-23, TNF, CXCL1/KC, and CXCL2/MIP-2) [13,93]. In macrophages and