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# Pattern recognition receptors and their role in invasive aspergillosis

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**Pattern recognition receptors (PRRs) are germline receptors that recognize conserved structures on microorganisms. Several PRRs have been identified in the recent years that are involved in the immune response against *Aspergillus fumigatus*. The role of PRRs in invasive pulmonary aspergillosis becomes especially apparent in the setting of an immunocompromised status of the host because of the redundancy of many PRRs in the host defense against *A. fumigatus*. Studies that investigated the PRRs and their effector pathways in invasive aspergillosis have led to a better understanding of the pathogenesis of invasive aspergillosis in immunocompromised patients. This knowledge may pave the way for novel diagnostic and immunomodulatory treatment strategies that are needed to overcome the high mortality associated with invasive *A. fumigatus* infection in immunocompromised patients.**

**Keywords:** *Aspergillus fumigatus*; Toll-like receptors; C-type lectin receptors; NOD-like receptors; innate immune cells

## Introduction

*Aspergillus fumigatus* can cause invasive pulmonary aspergillosis in immunocompromised patients. The management of invasive aspergillosis still presents a major challenge, and knowledge of the host defense against *A. fumigatus* is essential to develop new treatment strategies. Here, we review the literature on pattern recognition receptors (PRRs) that recognize *A. fumigatus,* and focus on their role in the host defense against invasive pulmonary aspergillosis (IPA).

## Pattern recognition of *A. fumigatus*

The first step in the innate host defense against *A. fumigatus* is the recognition of fungal cell wall components by PRRs present on and in the cells of the innate immune system. Three of the four major families of PRR have been implicated in recognition of *Aspergillus spp*: Toll-like receptors (TLRs), C-type lectin receptors (CLRs), and nucleotide oligomerization domain (NOD)–like receptors (NLRs) (Fig. 1). RigI helicases, the fourth PRR family, are not currently known to be involved in *A. fumigatus* recognition.

## Toll-like receptors

Both TLR2 and TLR4 signaling are associated with NF-κB translocation and production of proinflammatory cytokines in response to *A. fumigatus in vitro*.[1,2] Initial studies have shown that peritoneal macrophages deficient in TLR2 and MyD88 stimulated with *A. fumigatus* produce significantly lower TNF-α levels, whereas TLR4-deficient peritoneal macrophages produce normal levels of TNF-α when stimulated with the fungus.[3] Blocking TLR4, but not TLR2, resulted in decreased *A. fumigatus*–induced TNF-α production by human adherent monocytes.[4] Furthermore, conidia and hyphae of *A. fumigatus* stimulate cytokine production in macrophages through TLR2, whereas only conidia are able to stimulate macrophages by TLR4.[5] These *in vitro* studies are the first reports that have demonstrated a role for TLRs in the recognition of *A. fumigatus*. Most of these *in vitro* studies controlled for the absence of bacterial LPS.

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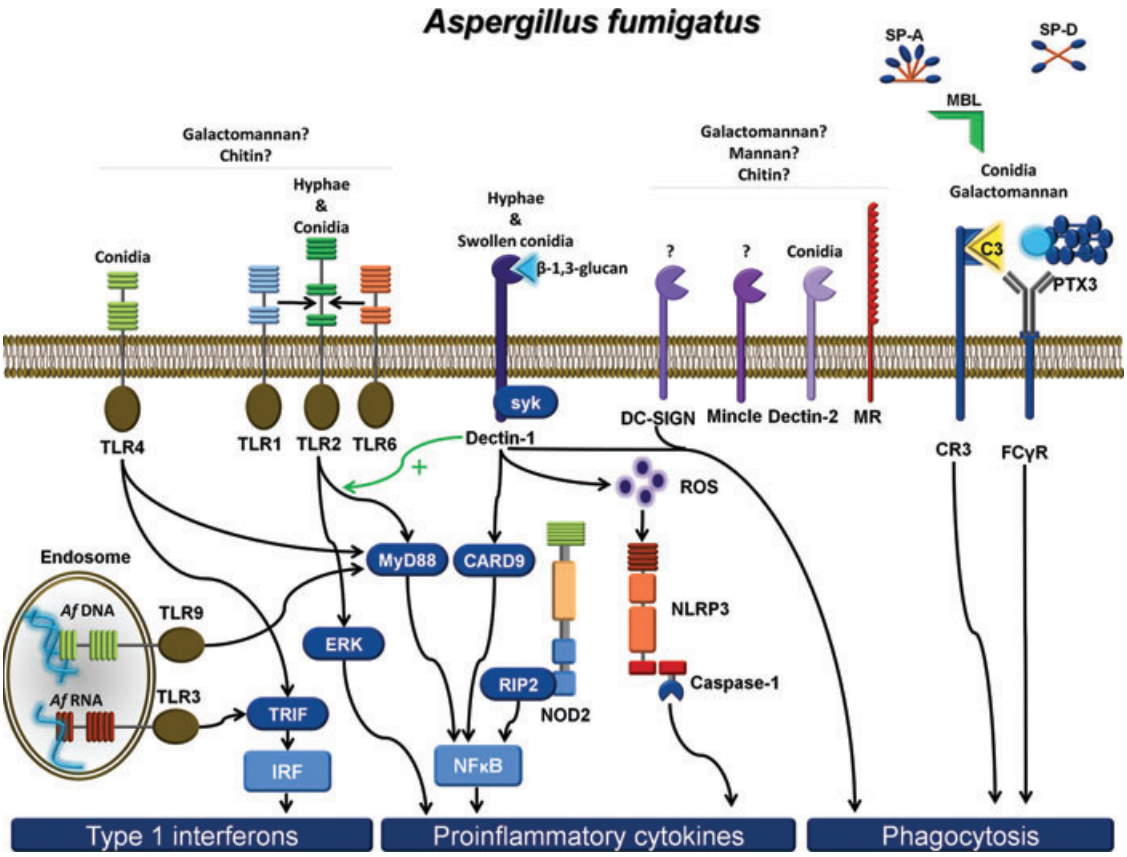


**Figure 1.** Schematic overview of the PRRs and the downstream signaling pathways involved in the recognition of *A. fumigatus*. Pathogen-associated molecular patterns (PAMPs) of *Aspergillus* lead to various host responses, such as cytokine production and phagocytosis through the activation of PRRs. Syk (spleen tyrosine kinase), DC-SIGN (dendritic cell-specific intercellular adhesion molecule-3-grabbing nonintegrin), MR (mannose receptor), CR3 (complement receptor 3), C3 (complement factor C3), SP-A/D (surfactant protein-A/D), PTX-3 (pentraxin-3), FCγR (FC gamma receptor), *Af* (*Aspergillus fumigatus*), TRIF (TIR-domain–containing adapter-inducing interferon-β), IRF (interferon-regulating factor), ERK (extracellular signal–regulated kinase), MYD88 (myeloid differentiation primary response gene 88), CARD9 (caspase recruitment domain–containing protein 9), RIP2 (receptor interacting protein 2), NF-κB (nuclear factor kappa light chain-enhancer of activated B cells), NOD2 (nucleotide-binding oligomerization domain–containing protein 2), NLRP3 (NACHT, LRR, and PYD domain–containing protein 3), ROS (reactive oxygen species).

In a murine model of IPA, TLR2, and TLR4 deficiency are both correlated with decreased cytokine levels, and TLR2$^{-/-}$, TLR4$^{-/-}$, or MyD88$^{-/-}$ mice have a higher fungal burden than wild-type (WT) mice.[6,7] TLR2 and TLR4 are important for the recruitment of PMNs to the lung during IPA, and the optimal killing of *A. fumigatus in vivo* requires both TLR2 and TLR4.[7] TLR2 can form heterodimers with TLR1 or TLR6, and a recent study has reported that TLR1$^{-/-}$ and TLR6$^{-/-}$ mice have increased fungal burdens during *A. fumigatus* infection. It has been shown that TLR1$^{-/-}$ and TLR6$^{-/-}$ macrophages produce fewer proinflammatory cytokines compared to wild-type mice.[8]

The intracellular TLRs, TLR3, and TLR9 have also been reported to play a role in anti-*Aspergillus* host defense. Epithelial cells can contribute to the protection against *A. fumigatus* in a TLR3-dependent pathway.[9] TLR3 signals through the adaptor molecule TRIF, and TRIF$^{-/-}$ mice are highly susceptible to invasive pulmonary aspergillosis. Furthermore, *A. fumigatus* RNA is recognized by TLR3, and dendritic cells can promote protective cytotoxic T cell responses through a TLR3-mediated pathway.[9] In line with these findings, TLR3$^{-/-}$ mice are highly susceptible to invasive pulmonary aspergillosis.[9] TLR9 can recognize *A. fumigatus* DNA,[10] and TLR9 is actively being recruited to phagosomes that

contain *A. fumigatus* conidia.[11] Interestingly, but still largely unexplained, is the finding that TLR9$^{-/-}$ mice are protected against lethal *A. fumigatus* infection.[7]

*A. fumigatus* itself can influence the immune response by modulating TLR signaling. *A. fumigatus* conidia can decrease TLR2 expression on the surface of monocytes by inducing TLR2 internalization, while *A. fumigatus* hyphae can selectively downregulate the TLR4-mediated immune response.[12] Moreover, the *Aspergillus* cell wall components β-glucan and galactomannan suppresses TLR4-induced responses, while α-glucan inhibits TLR2- and TLR4-induced IL-6 production.[13] TLR5 is strongly upregulated by *A. fumigatus* conidia in human monocytes, and although there is no evidence for direct recognition of *A. fumigatus* by TLR5, activation of TLR5 results in decreased capacity of monocytes to inactivate viable *A. fumigatus* conidia.[14] This suggests that the increased expression of TLR5 induced by *A. fumigatus* on the surface of human monocytes can help survival of the fungus when ligands are present that activate TLR5. However, all TLR5 ligands known to date are of bacterial origin, and TLR5 is most likely influencing host defense against *Aspergillus* in the context of cocolonization or coinfection with bacteria.

In line with the *in vitro* and *in vivo* data demonstrating an important role for TLRs in anti-*Aspergillus* host responses, several studies have reported polymorphisms in TLRs that are associated with invasive aspergillosis in patients. A single nucleotide polymorphisms (SNP) in TLR4 that influences TLR4 function is associated with invasive aspergillosis in patients that received hematological stem cell transplantation (HSCT).[15,16] Although, functional studies have demonstrated involvement of TLR2 in the recognition of *A. fumigatus,* to date no polymorphisms in TLR2 have been associated with increased susceptibility to *A. fumigatus* infection. However, SNPs in TLR1 and TLR6, coreceptors that form heterodimers with TLR2, are associated with the development of invasive aspergillosis.[17] A TLR3 SNP has been reported to be associated with increased risk of IPA in a cohort of patients with HSCT.[9]

## C-type lectin receptors

The extracellular portion of CLRs consists of several carbohydrate recognition domains, which enable binding to sugar moieties present on microorganisms. Dectin-1 recognizes β-1,3-glucans and is involved in the recognition of *A. fumigatus.*[18] Dectin-1 binding to germ tubes augments TLR2-mediated production of proinflammatory cytokines.[19] Alveolar macrophages induce strong inflammatory responses to swollen and germinating conidia, which correlates with the increased surface expression of β-glucan during germination.[20] The importance of Dectin-1 for anti-*Aspergillus* host defense has been demonstrated by the fact that Dectin-1–deficient mice are more susceptible to invasive pulmonary aspergillosis.[18,21] *A. fumigatus* induces the cytokines IL-17 and IL-22, important for neutrophil recruitment and the production of defensins by epithelial cells, respectively, in a Dectin-1–dependent way.[22–24] It must, however, be noted that the current literature describes both protective and detrimental effects of IL-17 in the host, depending on the host conditions in which the *Aspergillus* infection occurs.[21,24,25] Human airway epithelium and dendritic cells also express Dectin-1, which enables them to recognize *A. fumigatus*.[26] Human monocytes with the Dectin-1 Tyr238X polymorphism that results in an early stop codon produce fewer proinflammatory cytokines in response to *A. fumigatus* stimulation. Importantly, this polymorphism correlates with an increased susceptibility to develop invasive aspergillosis in patients receiving HSCT.[26,27] Recently, a study demonstrated increased susceptibility to invasive aspergillosis in hematological patients that carried SNPs in Dectin-1 intronic regions.[28]

DC-SIGN is a CLR that is expressed on dendritic cells (DCs) and macrophages, and has been shown to be involved in binding and internalization of *A. fumigatus*.[29] Costimulation of neutrophils with *Aspergillus*-stimulated DCs results in the upregulation of costimulatory molecules on DCs, a process that is dependent on DC-SIGN.[30] Notably, SNPs in intronic regions or in the 3′-UTR of the DC-SIGN gene have been associated with increased susceptibility to invasive aspergillosis.[28] Another CLR, the mannose receptor (MR) has been suggested to be involved in the induction of proinflammatory cytokine production induced by *A. fumigatus* conidia that are deficient in melanin.[31] Dectin-2 mediates the release of cysteinyl leukotrienes from murine bone marrow-derived DCs that are stimulated with extracts from *A. fumigatus*, suggesting that Dectin-2 recognizes *A. fumigatus.*[32] However, the function

and the importance of the MR and Dectin-2, as well as the role of other CLRs in anti-*Aspergillus* host defense, such as Mincle or macrophage galactose lectin, remains to be determined.

## NOD-like receptors

In addition to the TLRs and CLRs, NOD-like receptors (NLRs) that are localized intracellularly have been reported to be involved in the recognition of *Aspergillus*. NLRs are generally subdivided in two categories: NOD1 and NOD2 are the main intracellular receptors for peptidoglycans, whereas the function of the members of the NLRP subfamily is mainly in the formation of the inflammasome. NOD2 is upregulated after exposure to *A. fumigatus*, and recently it was reported that NOD2 recognizes *A. fumigatus,* which subsequently results in the activation of NF-κB.[33] However, individuals completely deficient in NOD2 and suffering from Crohn's disease[34] do not show an increased susceptibility to invasive aspergillosis.

NLRP3 is an NLR that has been extensively studied over recent years. NLRP3 is involved in the activation of the inflammasome, which is a protein complex that can activate caspase-1. Caspase-1 is an enzyme that can cleave the inactive protein pro-IL-1β into the active cytokine IL-1β. *A. fumigatus* hyphae, but not conidia, can activate the NLRP3 inflammasome through the induction of reactive oxygen species (ROS) and potassium efflux.[35] This, in turn, results in the release of active IL-1β. This process is dependent on Syk, which is an adaptor molecule downstream of Dectin-1, suggesting that Dectin-1 is involved in the induction of ROS, the activation of the NLRP3 inflammasome, and release of IL-1β induced by *A. fumigatus*.

## Soluble pattern recognition receptors

Collectins are soluble C-type lectins that bind to glycoconjugated structures. Surfactant proteins A (SP-A) and D (SP-D) are collectins that are produced by pneumocytes and that can bind to *A. fumigatus* conidia. The survival of immunosuppressed mice infected with *A. fumigatus* is significantly increased when mice are treated with SP-D.[36] In line with this, SP-D$^{-/-}$ mice die earlier than WT mice, and treatment of SP-D$^{-/-}$ mice with SP-D decreases mortality from 50% to 33%.[37] Interestingly, SP-A$^{-/-}$ mice are less susceptible to invasive aspergillosis and SP-A treatment increases mortality in SP-A$^{-/-}$ mice.[37]

Although mannose-binding lectin (MBL) can bind to *A. fumigatus,* this does not necessarily result in enhanced killing of *A. fumigatus*.[38] Immunocompetent mice deficient in MBL are protected against invasive aspergillosis.[39] In contrast, immunosuppressed mice with invasive aspergillosis that are treated with recombinant MBL have a clear survival benefit.[38] Thus, MBL could have different effects depending on the status of the immune system. In addition, low MBL concentrations in serum are associated with higher susceptibility to invasive aspergillosis.[40]

Pentraxin 3 (PTX3) is a soluble pattern recognition receptor that can bind to *Aspergillus* conidia, but not to hyphae.[41] PTX3-deficient neutrophils display impaired phagocytosis of *Aspergillus.*[41] DCs and alveolar macrophages can recognize *A. fumigatus* through a PTX3-dependent pathway, and genetic deletion of PTX3 in mice results in impaired recognition of *A. fumigatus* and increased susceptibility to *A. fumigatus* infection.[41] The complement receptor 3 (CR3) and the FCγR are required for recognition of PTX3-opsonized *Aspergillus.*[42] Notably, NADPH-dependent ROS-deficient mice[43] and corticosteroid immunosuppressed rats,[44] which are both highly susceptible to *Aspergillus* infection, can be rescued by PTX3 treatment. Therefore, PTX3 treatment can prove to be an important immunotherapeutic strategy in invasive aspergillosis.

## Pattern recognition receptors orchestrate the immune response

When *A. fumigatus* conidia are inhaled and reach the alveolus, PRRs present on alveolar macrophages and epithelial cells, and the soluble PRRs from the alveolar fluid will recognize the fungus and induce the activation of the immune response (Fig. 2). Alveolar macrophages and epithelial cells will release cytokines and chemokines in response to *A. fumigatus* recruiting innate immune cells, a process dependent on TLR2, TLR4, and Dectin-1.[1–7] Both neutrophils and macrophages recognize and phagocytose *A. fumigatus* conidia by a process that can be enhanced by soluble PRRs such as PTX3 and, possibly, SP-D and MBL;[36,38,42] neutrophils and macrophages can also kill conidia by NADPH-dependent ROS production.[45,46] *A. fumigatus*–induced ROS production can also

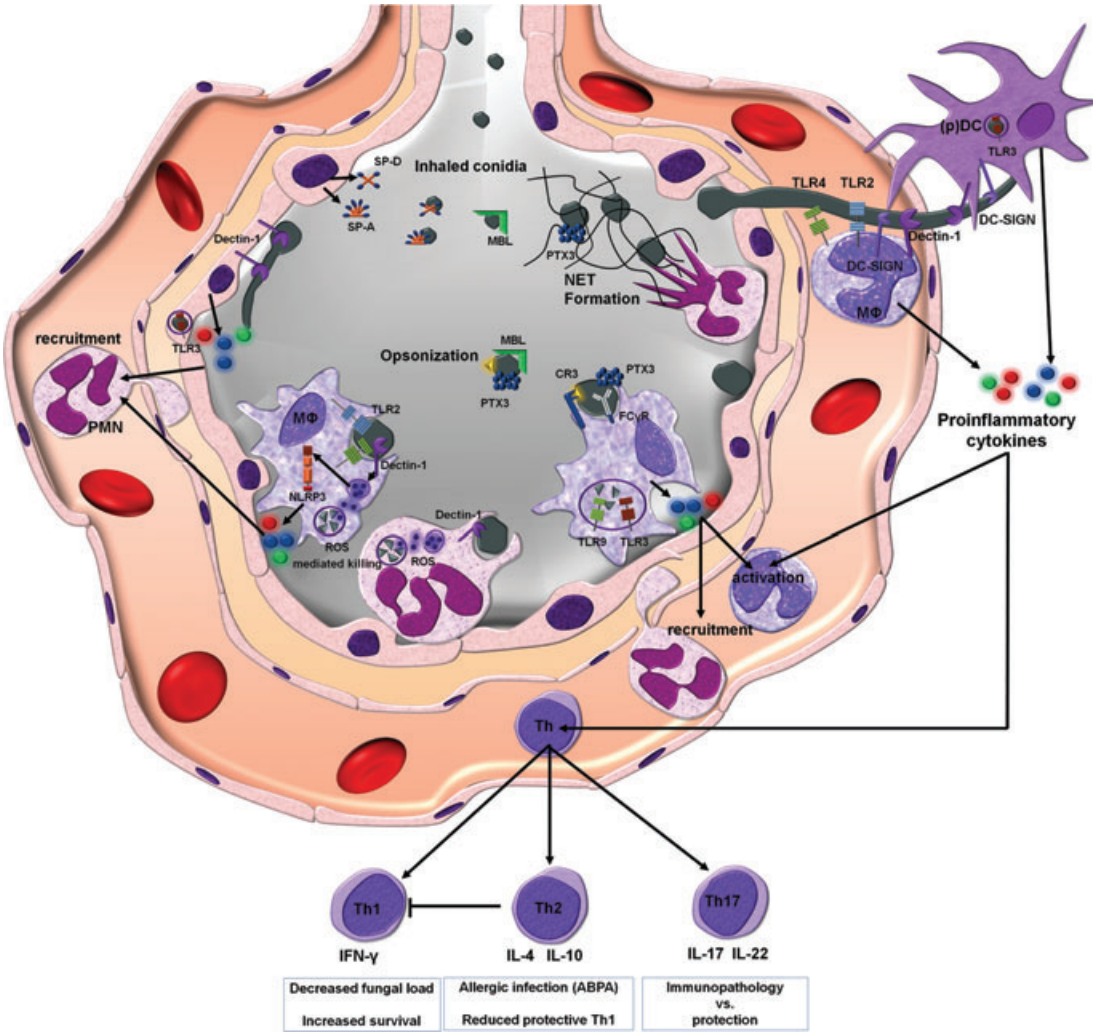


**Figure 2.** Overview of the PRR-mediated effector functions of innate immune cells that play a role in the pulmonary antifungal host defense against *A. fumigatus*. Inhaled conidia are recognized and opsonized by soluble PRRs-like pentraxin 3 (PTX3), mannose binding lectin (MBL), and surfactant protein-A/D (SP-A and SP-D). Epithelial cells and macrophages (Mϕ) produce cytokines through Toll-like receptors (TLRs) that mediate recruitment of innate immune cells like neutrophils (PMNs) and dendritic cells (DCs). Following internalization by dectin-1, phagocytes induce reactive oxygen species (ROS) to mediate eradication of the internalized conidia. Neutrophils generate neutrophil extracellular traps (NETs) by releasing their intracellular contents and DNA. DC-SIGN (dendritic cell–specific intercellular adhesion molecule-3–grabbing nonintegrin), CR3 (complement receptor 3), FCγR (FC gamma receptor), NLRP3 (NACHT, LRR, and PYD domain–containing protein 3).

trigger the NLRP3 inflammasome through Dectin-1 /Syk signaling, resulting in the release of active IL-1β and recruitment of neutrophils.[35] Neutrophils will release neutrophil entrapment traps (NETs) that, in turn, might help to kill and inhibit growth of *A. fumigatus* hyphae that will be too large to be phagocytized.[47,48] Notably, restoration of NADPH oxidase activity by gene therapy in patients with chronic granulomatous disease provides protection against invasive aspergillosis.[49,50] The restoration of the NADPH oxidase activity restores NET formation and calprotein release, which is associated with more efficient killing of *Aspergillus ex vivo*.[49,50] Recently, plasmocytoid DCs have been reported to have the ability to kill hyphae extracellularly.[51] However, the PRRs involved in this process, and the

PRRs that trigger the formation of NETs in response to *Aspergillus* have not yet been elucidated. If the innate immune system fails to control the infection, antigen-presenting cells such as dendritic cells will activate the adaptive immune response, a process where Dectin-1 and TLR3 play an important role.[9,52]

The interplay between other CLRs and TLR-MyD88 pathways are also important for the induction of the adaptive immune response. Especially Dectin-1, which can regulate the balance between Th1 and Th17 responses.[53] Although TLR-MyD88 signaling is crucial for IFN-γ production in *Aspergillus*-specific CD4 T cells, it is not required for the recruitment or proliferation of these cells in response to *A. fumigatus*.[54] The TLR-MyD88 pathway plays a less important role in the induction of Th2 responses by *A. fumigatus*,[54] in contrast to the induction of Th2 cells by OVA, which is MyD88 dependent.[55]

## Concluding remarks

Although PRRs play an important role in the host defense against *A. fumigatus*, it has to be noted that invasive *A. fumigatus* infections only occur in immunosuppressed patients. MyD88$^{-/-}$ mice only develop invasive aspergillosis when they are immunosuppressed, and nonimmunosuppressed patients with MyD88 deficiency are not prone to develop invasive pulmonary aspergillosis.[56,57] Furthermore, humans that lack surface expression of Dectin-1, which is involved in many fundamental anti-*Aspergillus* host responses, do not suffer from invasive aspergillosis when they are not immunosuppressed. These findings suggest that the TLR and Dectin-1 pathways are redundant in anti-*Aspergillus* host defense. On the one hand, this suggests that only concomitant deficiencies in more recognition pathways at the same time will likely result in an increased susceptibility, and this hypothesis is supported by the increased susceptibility to infections in CARD9-deficient patients, who display defects in several CLR pathways, and possible also NOD2-dependent signaling.[58] On the other hand, invasive aspergillosis especially develops in patients that are immunosuppressed, and it is in this particular setting that polymorphisms and deficiencies in TLR, Dectin-1, and other PRR signaling pathways become highly relevant for the antifungal host defense.

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## Conflicts of interest

The authors declare no conflicts of interest.

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