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FUNGAL GENOMICS AND PATHOGENESIS (S SHOHAM, SECTION EDITOR)

Genetic Regulation of the Host-Fungus Interaction in the Pathogenesis of Aspergillosis

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Abstract

Purpose of Review There is significant interindividual variability in the development and progression of fungal diseases, most notably invasive pulmonary aspergillosis (IPA). The integration of individual traits into clinically valid procedures to predict the risk and progression of infection, and the efficacy of antifungal prophylaxis and therapy, will change the current healthcare landscape regarding the management of patients at risk of IPA and, likely, other fungal infections.

Recent Findings Over the last decade, an expanding number of common polymorphisms associated with IPA have been reported, adding to the information available on monogenic defects underlying severe forms of the disease. Predisposition to IPA is therefore nowadays considered to result from a combination of clinical and host factors, with the latter being most likely regulated at the genetic level.

Summary In this review, we address the contribution of the genetic profile of the host to the outcome of the host-fungus interaction and discuss the application of this information in potential strategies with the aim of moving towards personalized prognostics, diagnostics, and treatment.

Keywords Aspergillosis · Immunocompromised host · Single nucleotide polymorphism (SNP) · Genetic susceptibility · Antifungal immunity · Personalized medicine

Introduction

Invasive pulmonary aspergillosis (IPA) is a life-threatening infection caused primarily by the opportunistic fungal pathogen *Aspergillus fumigatus* [1]. This disease commonly affects patients with impaired immune function, including those undergoing hematopoietic stem cell (HSCT) or solid organ transplantation (SOT) and cancer therapy, or with selected primary immunodeficiencies [2]. Because of the severe underlying immune dysfunctions, including neutropenia or NADPH oxidase activity, IPA is typically associated with high mortality rates. Other groups of patients with less severe or altogether lacking apparent immunosuppression are at risk of non-invasive fungal colonization. These include patients with underlying pulmonary complications, such as cystic fibrosis and chronic obstructive pulmonary disease (COPD), which are prone to develop allergic bronchopulmonary aspergillosis (ABPA) or chronic pulmonary aspergillosis (CPA), respectively [3].

The risk of fungal infection and its clinical outcome vary considerably even among patients with similar predisposing clinical conditions and microbiological exposure. Since there is currently no robust evidence for geographical or genomic factors influencing the virulence of *A. fumigatus*, susceptibility to infection is thought to depend mainly on genetic predisposition and the degree of pathogen exposure, with interactions between the two likely contributing substantially to the risk of infection [4–6]. Accordingly, studies in mice have revealed disparate susceptibility profiles to experimental aspergillosis between inbred strains [7], reflecting a marked contribution of heritable factors to the development of infection. Importantly, the study of individuals with rare monogenic defects and from common single nucleotide polymorphisms

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(SNPs) in cohort-based studies have pinpointed defined molecular players and mechanisms of genetic control of the antifungal immune response [8••]. Here, we review the relevant contribution of common human genetic variation to antifungal immunity and the mechanisms through which it predisposes to infections caused by *A. fumigatus* (Fig. 1).

Genetic Regulation of Innate Immunity to *Aspergillus*

Epithelial Immunity

The lung epithelium is the initial site of the host-fungus interaction, and recent work has underscored its critical role in defining progression from relatively innocuous colonization to overt disease [9]. Despite its importance, not much is known directly implicating epithelial immunity with the development of aspergillosis. One exception was provided recently in a study involving patients with ABPA and in which a SNP in the zinc finger protein 77 (ZNF77), whose function is required for normal epithelial integrity, was found to predispose to enhanced fungal colonization of the lungs [10•]. Additional studies in bronchial epithelial cells whose genome was edited to harbor the risk genotype revealed a decreased epithelial integrity, with fungal conidia adhering and germinating more efficiently as the result of enhanced synthesis of adhesive extracellular proteins. Further recent studies have highlighted additional mechanisms through which epithelial immunity controls the initial stages of infection and how the fungus has instead evolved strategies to counter these mechanisms of epithelial control [11, 12]. However, it remains to be assessed whether these mechanisms of resistance are influenced by host genetic variation and how they contribute to human disease.

Pattern Recognition Receptors

Innate immune recognition of fungal cell wall components has been an area of intense research, and multiple families of pattern recognition receptors (PRRs), including C-type lectin receptors (CLRs) and Toll-like receptors (TLRs), are known to mediate the recognition of fungal cell wall components [13•]. Functionally, these PRRs induce the secretion of proinflammatory cytokines and chemokines and activate mechanisms leading to phagocytosis and production of reactive oxygen species (ROS), as well as the activation of complex immunoregulatory processes and adaptive immunity [14]. The efficiency of fungal recognition and the interaction with membrane-associated PRRs also relies largely on the opsonization by different soluble pattern recognition molecules (PRMs), including collectins, pentraxins, ficolins, and components of the complement pathway [15]. Given their pivotal role in innate antifungal immunity, it is not surprising that genetic variation in these genes constitutes major risk factors to diseases caused by a wide range of fungal pathogens [16].

Toll-Like Receptors

The TLR family is endowed with prominent genetic variability due to the strong selective pressure occurring during evolution [17]. Given the high number of polymorphic sites in the coding regions from these genes, TLRs were historically considered plausible targets for involvement in susceptibility to infectious diseases, including fungal infections [18]. The presence of a haplotype in the leucine-rich repeat region of TLR4

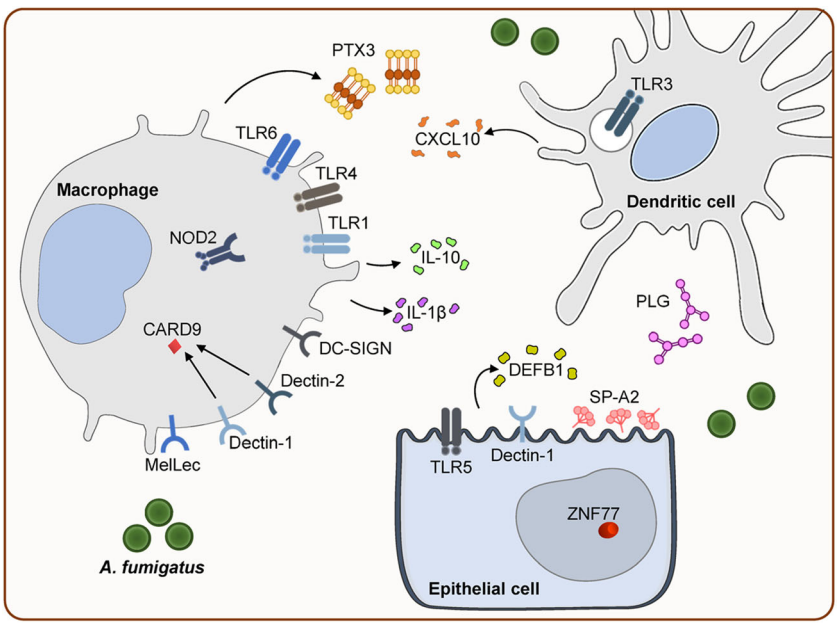

Fig. 1 Overview of the major innate immunity components and the cell types in which genetic defects increase susceptibility to different forms of aspergillosis. DC-SIGN, dendritic cell-specific intercellular adhesion molecule-3-grabbing non-integrin; DEFB1, β-defensin 1; CARD9, caspase recruitment domain-containing protein 9; CXCL10, C-X-C motif chemokine 10; NOD2, nucleotide-binding oligomerization domain-containing protein 2; PLG, plasminogen; PTX3, pentraxin-3; SP-A2, surfactant protein A2; TLR, Toll-like receptor; ZNF77, transcription factor zinc finger protein 77

in allogeneic HSCT donors was associated with the development of IPA in the corresponding recipients [19], a finding that was validated in independent HSCT cohorts [20, 21] and in immunocompetent individuals suffering from CPA [22], but that failed to be replicated in other studies [23, 24••, 25]. These discrepant findings may be related in part to the yet unknown mechanisms through which TLR4 variants may influence antifungal immune responses, particularly since no fungal ligand for TLR4 has been identified to date.

Although SNPs in additional TLRs have been reported to influence susceptibility to IPA, most of these studies involved small sample sizes and failed to provide any functional validation [26, 25]. One relevant exception regards a study implicating a regulatory variant in TLR3—the prototypical receptor for double-stranded RNA—in the development of IPA after HSCT [27], an association that was replicated in patients suffering from severe asthma with fungal sensitization [28]. Although definitive data demonstrating direct binding of fungal RNA to TLR3 has yet to be provided, dendritic cells from SNP carriers displayed an impaired expression of TLR3, resulting in a defective priming of memory CD8(+) T cell responses to the fungus [27]. Such functional elucidation provided pivotal evidence of how genetic defects in PRRs may influence adaptive immune responses, in addition to fungal sensing and innate immunity. Importantly, as host damage perception is also fundamental for resolution of infection, genetic variants triggering a hyperactivation of damage-associated molecular pattern signaling, and presumably leading to uncontrolled inflammatory response to the fungus, were also found to increase the risk of IPA among HSCT recipients [29].

C-Type Lectin Receptors

The CLR family includes the receptors with the most well-established roles in the coordination of antifungal immune responses [30]. For example, the importance of dectin-1 in the recognition of β-1,3-glucan and activation of antifungal immunity has been revealed in mouse studies and confirmed in patients with recurrent fungal infections carrying the early stop codon SNP Y238X [31, 32]. This SNP results in a truncated form of dectin-1 lacking several amino acids within the carbohydrate recognition domain, with a detrimental effect on recognition of β-1,3-glucan and cytokine production in response to fungal stimulation [33•, 31]. As a result, Y238X was also found to predispose HSCT recipients to the development of IPA in different cohorts [34, 33•]. Additional variants in dectin-1, but also dectin-2 and DC-SIGN (CD209), were likewise correlated with the development of IPA in hematological patients [35, 36]. Importantly, dectin-1 deficiency in both donors and recipients of HSCT was found to display a concerted action towards the risk of infection [33]. This finding was replicated in a large, independent HSCT patient cohort [24••], highlighting the key role of dectin-1 in antifungal immunity across multiple cell types. Because the spatial localization of the different dectin-1 isoforms within the cell orchestrates the signaling quality of antifungal immune responses [37, 38], it is also tempting to speculate that this may be one additional mechanism through which the Y238X variant contributes to infection.

The role of dectin-1 signaling in antifungal immunity may however extend beyond the direct activation of effector mechanisms. For example, recognition of β-1,3-glucan is nowadays acknowledged as one major mechanism involved in the establishment of innate immune memory to infection with a broad range of pathogens—a process referred to as trained immunity [39]—and that occurs through the regulation of multiple processes of cellular metabolism and epigenetic regulation [40, 41, 42••, 43]. Whether the Y238X SNP also contributes to infection by compromising the induction of "natural" trained immunity as the result of our constant exposure to fungi remains to be assessed. Either way, this may prove to be a target amenable to therapeutic manipulation, particularly in the context of fungal diseases.

In addition to dectin-1, MelLec was recently identified as another critical CLR activated in response to *A. fumigatus* [44••]. MelLec recognizes melanin in the cell wall of dormant conidia and is required for the induction of protective immunity to the fungus. Importantly, human monocyte-derived macrophages from carriers of a non-synonymous SNP affecting the cytoplasmic tail of MelLec, and likely impacting intracellular signal transduction, were found to display a generalized defect in the production of cytokines after fungal stimulation. As a result, HSCT patients that received transplants from donors carrying the SNP displayed a markedly increased risk of IPA after transplantation. Besides the role of melanin as a major determinant of the cell wall interaction with the innate immune system, it endows the fungus with the ability to survive killing by phagocytes, namely, by blocking phagosome biogenesis, a mechanism that depends on calcium sequestration inside the phagosome [45, 46•]. Importantly, disruption of these protective mechanisms by the presence of a regulatory variant in the gene encoding calmodulin 1, likely affecting calcium signaling, increased the risk of IPA [46•]. Although the mechanisms through which MelLec and melanin orchestrate antifungal immunity are still incompletely understood [47], these findings nevertheless support the concept that CLRs are important repositories of genetic variability regulating susceptibility to IPA.

Caspase recruitment domain-containing protein 9 (CARD9) is an adaptor molecule that transduces signals from dectin-1 and other CLRs and whose deficiency was initially identified in patients suffering from mucocutaneous fungal infections [48]. More recently however, human CARD9 deficiency was also found to predispose to extrapulmonary aspergillosis through a mechanism involving the defective accumulation of neutrophils in the infected tissue [49]. In addition to

these rare mutations, functional studies in vivo using knock-in mice carrying the common S12N SNP have implicated common variation in CARD9 in the pathogenesis of ABPA [50]. Mechanistically, S12N contributed to the activation of NF-κB subunit RelB, which in turn promoted the production of IL-5 by alveolar macrophages and the recruitment of eosinophils to drive Th2 cell-mediated allergic responses. Given the central role of CARD9 in collecting signals from the CLR family and orchestrating signals driving antifungal immunity, it is tempting to speculate that this or other common genetic variants in CARD9 may also be relevant in patients at risk of other forms of aspergillosis and eventually other fungal infections.

NOD-Like Receptors

Besides the involvement of nucleotide-binding oligomerization domain (NOD)-like receptors (NLRs) in the formation of inflammasomes, oligomeric cytosolic structures known to play a key role in fungal sensing and immunity [51,52], the function of canonical NLRs such as NOD1 and NOD2 in host defense against fungi remained until recently poorly studied. The P268S SNP in NOD2, typically associated with inflammatory diseases, such as Crohn's disease [53], was found to confer resistance to IPA in HSCT patients [54•]. Mechanistically, mononuclear cells harboring this SNP displayed enhanced phagocytosis and killing capacity as the results of a compensatory mechanism leading to the increased expression of dectin-1, a finding that was corroborated in a mouse model of infection using NOD2-deficient mice. This variant illustrates therefore how genetic variation can influence the intricate crosstalk between innate immune receptors and highlights the possibility to disrupt NOD2 signaling as a therapeutic intervention in IPA.

Pattern Recognition Molecules

As referred above, there are several circulating molecules that are endowed with the ability to interact with and bind to microbial polysaccharides without transducing intracellular signals and that function as opsonins to facilitate recognition and phagocytosis [15]. One classical example regards the mannose-binding lectin (MBL), a CLR that binds carbohydrate patterns from microorganisms and activates the lectin pathway of the complement system [55]. There are several described combinations of non-synonymous and promoter variants in the gene encoding MBL, either affecting the expression levels, its functional activity, or both [16]. Although there is no evidence for a contribution of genetic variants in MBL to IPA, low circulating concentrations of the protein were detected in infected patients [56]. Instead, the development of CPA was nonetheless linked with the presence of variable MBL alleles [57, 58]. Other studies have also implicated SNPs in lung surfactant proteins, such as SP-A2, in ABPA [59, 60]. However, most of these studies were limited by the small sample size, and these associations need to be revisited in larger and well-characterized cohorts.

Another PRM that has received a great deal of recent attention in the field of fungal diseases is the long pentraxin-3 (PTX3) [55]. This molecule binds microbial moieties from a wide range of microorganisms, including bacteria, viruses, and fungi, particularly *A. fumigatus* [61]. Accordingly, genetic variation in PTX3 was identified as a major risk factor for IPA after HSCT [62••], an association that was validated in independent cohorts of recipients of HSCT [24••] and SOT [63, 64] and patients with COPD [65, 66]. Mechanistically, genetic variants in PTX3 were found to compromise the normal expression of the protein in the lungs and, at a cellular level, the antifungal effector mechanisms of neutrophils were impaired [62••]. The specific impact of PTX3 deficiency on neutrophil function was corroborated by a recent study describing the same association in patients with acute myeloid leukemia undergoing chemotherapy courses without pre-existing neutropenia [67]. Collectively, these studies support variation in PTX3 as the most robust genetic marker for IPA identified to date and lay the foundations for prospective clinical trials assessing their prognostic performance in the clinical setting.

Other mechanisms of antifungal host defense besides neutrophil function may also be regulated by PTX3 and influenced by its deficiency. For example, PTX3 has been shown to bridge neutrophil and B cell functions in the spleen, namely, class switching, plasmablast expansion, and antibody production [68]. As such, antibodies against fungal antigens could be compromised by PTX3 deficiency, and this could represent one additional mechanism potentially explaining the association with increased susceptibility to IPA. In addition, PTX3 was found to directly bind to myeloid differentiation protein 2 (MD-2), an adapter of the TLR4 signaling complex, and this process was found to be critically required for immune protection in experimental aspergillosis [69]. This raises the possibility that the combined genetic deficiency of PTX3 and TLR4 might underlie a higher risk of IPA than the single defects alone, a hypothesis that requires further confirmation.

The clinical applicability of PTX3 in the setting of aspergillosis may also extend beyond risk stratification approaches. Levels of PTX3 in the bronchoalveolar fluids were proposed to be relatively accurate diagnostic markers for microbiologically confirmed pneumonia [70]. Because the concentrations of PTX3 in each individual are determined at the genetic level [62••], one can predict an improvement to the diagnostic performance of PTX3 by stratifying patients according to their

genotypic profile for PTX3. In addition, PTX3 deficiency was also shown to impair the levels of alveolar cytokines, namely IL-6 and IL-8, in hematological patients suffering from IPA and to impact their ability to act as discriminators of infection [71]. Of note, because supplementing otherwise deficient neutrophils with recombinant PTX3 was sufficient to restore the efficacy of their antifungal effector functions [62••], the targeted administration of PTX3 can be regarded as a promising prophylactic or therapeutic approach for IPA in patients at risk [72].

Plasminogen represents another example of a PRM that displays relevant genetic diversity. By performing an unbiased screen of mice subjected to experimental aspergillosis with different strains and correlating genetic data with survival, a non-synonymous variant was associated with the risk of IPA in patients undergoing HSCT [73]. These findings support the importance of additional pre-clinical studies testing different models of infection and evaluating additional immune-related readouts to guide the discovery of human genetic variation with an important contribution to the risk of infection.

Cytokines and Chemokines

A number of positive associations between genetic variants in cytokines (e.g., IL-1 gene cluster, IL-12, IL-10, and IFN-γ) and chemokines (e.g., CXCL10) and susceptibility to aspergillosis have also been reported [74–78]. One of the most relevant examples available to date regards the immunoregulatory cytokine IL-10, which has been observed at elevated levels in patients with CPA [79] and in non-neutropenic patients with IPA [80]. In addition, significant relationships between genetic variation in IL-10 and aspergillosis have been found in patients with cystic fibrosis [81], hematological patients undergoing chemotherapy [76], and HSCT recipients [82]. The latter data was recently validated in a large, two-stage association study demonstrating the contribution of a specific variant in the IL-10 promoter to the risk of IPA, an association occurring, at least in part, due to a shift towards an anti-inflammatory cytokine profile in patients carrying IL-10 high-producing genotypes [83].

Other relevant reports have implicated genetic variation in IL-1β and beta-defensin 1 (DEFB1) in susceptibility to mold infection after SOT by affecting the production of proinflammatory cytokines by mononuclear cells [84]. In addition, a specific allele in the promoter of IFN-γ was recently found to confer resistance to IPA [75]. Although the exact molecular mechanisms though which this variant controlled risk of infection are not known, cells harboring the implicated allele displayed nonetheless an enhanced fungicidal activity. Regarding chemokines, one robust study implicated a haplotype in CXCL10 and risk of IPA in HSCT recipients [85]. Mechanistically, this haplotype was correlated with the inability of dendritic cells to express CXCL10, and, interestingly, patients who survived IPA displayed significantly higher CXCL10 levels compared with patients without the disease. Taken together, these observations suggest the need for evaluation of interindividual variability in immune function to assess the performance of novel diagnostic and immunotherapeutic approaches for aspergillosis [86].

Conclusions—Clinical Translation of Host Genetics

Early diagnosis remains critical to obtain a favorable outcome in patients suffering from aspergillosis. However, the existing tools are often compromised by slowness, invasiveness, lack of standardization, and insufficient understanding of their kinetics [87]. Given these technical barriers, the search for diagnostic tools that are more efficient and reliable is an active field of research. Although the interaction of the fungus with the immune system is being exploited to project novel and improved fungal diagnostics, efforts have on the other hand been also devoted to the implementation of clinical models aimed at the prediction of infection in high-risk patients. In this regard, interpretation of individual signatures associated with impaired antifungal immune responses and their integration with clinical data is regarded as a promising approach [88]. However, because the effect size of single variants may not be discriminatory enough to support clinical decisions, there is the need to account for a broader set of variants, likely impacting different, yet equally relevant, susceptibility mechanisms [6]. As a matter of fact, a recent study evaluating the combination of multiple genetic and clinical factors into a predictive model has demonstrated that such information could be used to successfully guide pre-emptive therapy in hematological patients [89].

Besides improved diagnostics, functional analyses of genetic variation known to impact susceptibility to infection remain a priority, since an improved understanding of the multiple targets and pathways affected by genetic variation may contribute to the establishment of innovative and personalized immunotherapeutics. This is illustrated by the pre-clinical evidence showing that genetic PTX3 deficiency can be rescued by the exogenous administration of the protein, a finding that may support its personalized use in specific patients at high risk of infection. In conclusion, the success of novel diagnostic and immunotherapeutic approaches for IPA and likely other fungal diseases will only be possible if guided by personalization based on the interindividual variability in antifungal immune function.

Compliance with Ethical Standards

Conflict of Interest Agostinho Carvalho reports that the work was supported by the Northern Portugal Regional Operational Programme (NORTE 2020), under the Portugal 2020 Partnership Agreement, through the European Regional Development Fund (FEDER) (NORTE-01-0145-FEDER-000013); the Fundação para a Ciência e Tecnologia (FCT) (IF/00735/2014 and CEECIND/03628/2017); and the European Society of Clinical Microbiology and Infectious Diseases (ESCMID Research Grant 2017). Cristina Cunha reports funding from the Fundação para a Ciência e Tecnologia (FCT) (PTDC/SAU-SER/29635/2017 and CEECIND/04601/2017) and the Institut Mérieux (Mérieux Research Grant 2017).

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- • Of importance
- •• Of major importance

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