Host genetics and invasive fungal diseases: towards improved diagnosis and therapy?

Invasive fungal diseases are life-threatening conditions affecting severely immunocompromised patients such as stem-cell transplant recipients. Recent concerns over antifungal prescription and the exceptionally high healthcare costs owing to the chronic course and high mortality rates of fungal diseases have been shifting the attention from universal antifungal prophylaxis to risk stratification and preemptive approaches [1]. This has motivated the search for ‘individual’ risk factors with prognostic value for the identification of patients most vulnerable to fungal diseases. Therefore, genetic screening strategies are expected to be successfully employed in the near future, to predict risk of disease and efficacy of antifungal therapies.

Genetic defects of the immune system & susceptibility to fungal diseases

A significant number of association studies linking genetic variants in the immune system and risk of invasive fungal diseases has been described [2,3]. One of the most important studies has disclosed a donor haplotype in TLR4 as a risk factor for invasive aspergillosis (IA) among hematopoietic stem cell transplant (HSCT) recipients [4]. Interestingly, the same polymorphisms had also been associated with susceptibility to pulmonary aspergillosis in immunocompetent individuals [5], thereby excluding potential confounders related to cytomegalovirus disease or antifungal prophylaxis. However, the prognostic value of TLR4 polymorphisms remains inconclusive, as a number of other studies have failed to replicate such findings [6,7]. It is likely that the contribution of genetic variants to IA may depend on the specific type of transplantation and/or associated demographic and clinical covariables. One additional concern regards the current lack of information on the biological implications of human TLR4 deficiency in the immune response to fungi. Such knowledge would further support the use of genetic screening for TLR4 polymorphisms in patients at risk, as well as allowing a better understanding of their clinical repercussions. Indeed, the fact that most of the recent studies in the field have undertaken considerable efforts to link genetic associations with the functional consequences of disease-associated variants, is noteworthy. For example, the increased susceptibility to IA observed among HSCT patients with a TLR3 polymorphism was reinforced by the finding that antigen-presenting dendritic cells carrying this variant were unable to efficiently prime protective CD8+ memory responses to the fungus [8]. Besides shedding light into the unsuspected role of TLR3 in the adaptive immune responses to fungi, these findings point to the usefulness of vaccinomic strategies for the design and application of antifungal vaccine candidates.

A pivotal role for the β-glucan receptor, dectin-1, as the prototype of the innate, non-TLR signaling pathway for antifungal sensing has been highlighted [9]. Accordingly, human dectin-1 deficiency has been recently found to contribute to susceptibility to IA...
Pathogen sensing combines host-damage perception, via damage-associated molecular patterns, and the receptor for advanced glycation end products in promoting inflammation and resolution of infection. Recently, inflammation in the setting of experimental aspergillosis was found to be restrained through a tight control of the danger molecule S100B [12]. In this regard, attention has also been directed to polymorphisms in genes involved in danger sensing. Accordingly, genetic variants in the S100B/the receptor for advanced glycation end products axis have recently been proposed to increase the risk to IA in HSCT recipients [13]. Functional assays demonstrated a hyperfunction of this signaling pathway, presumably underlying over-reactive inflammatory responses to the fungus, therefore contributing to disease susceptibility. Altogether, this study demonstrated the need to look beyond pattern recognition receptors as determinants of susceptibility to fungal diseases. Indeed, a few strategies have been used aiming to uncover unsuspected candidate genes involved in the susceptibility to IA. The genetic mapping analysis of the survival data of an immunocompromised mice model has also allowed the identification of plasminogen, a regulatory molecule that binds to the fungus, as a suitable candidate gene for aspergillosis susceptibility [14]. The clinical translation of these findings disclosed a nonsynonymous polymorphism in the human plasminogen gene to influence the risk of developing IA in HSCT recipients, particularly late after transplantation [14].

A number of studies have also addressed the role of genetic variants in cytokine genes in determining vulnerability to fungal diseases, particularly IA in non-HSCT patients. Although some positive associations have been reported [15], the lack of functional data precludes definite conclusions about the role of polymorphisms affecting cytokine production. On the other hand, one interesting study has reported an association between a haplotype in CXCL10 with susceptibility to IA in HSCT recipients [16]. Mechanistically, this haplotype was related with the inability of immature dendritic cells to trigger CXCL10 expression. In this regard, it is also worth mentioning that patients who survived IA had significantly higher CXCL10 levels in comparison to healthy controls.

**Genome-wide association studies: the road ahead**

Current information on the genetic basis of susceptibility to invasive fungal diseases is founded on the screening for single polymorphisms in candidate genes using small patient cohorts. Furthermore, no analysis of gene copy number variations as genetic risk factors has been performed so far. Given that an informative set of more than 1 million genetic markers across the genome can nowadays be used to carry out genome-wide association studies (GWASs), this approach may represent a valuable tool to use in the near future for identifying prognostic markers of predisposition to fungal diseases. One major advantage of GWAS over candidate gene approaches is the identification of genes and/or pathways involved in disease susceptibility that were otherwise unsuspected. However, besides the costs associated with high-throughput single nucleotide polymorphism genotyping, GWASs require a massive number of cases in order to subject the data to rigorous error detection and prevent loss of power, therefore making it impossible to perform at a single-center level. For this reason, a large multicenter GWAS is currently underway in Europe, which will hopefully provide the first results in the very near future. However, one should keep in mind that this approach does not diminish the importance of functional studies. Given that GWASs by nature ignore all prior knowledge about disease pathology, functional studies on the relevant markers found are essential in order to attribute a biological significance to the findings.

In conclusion, the identification of genetic markers predisposing to invasive fungal diseases in high-risk patients is currently one major priority in the field of hematology and microbiology. Given the efforts carried out aiming at the detection of accurate prognostic markers, it is expected that these may provide important implications regarding patient outcome and healthcare costs in the near future. In particular, as well as the identification of patients at highest risk for disease, these markers may also contribute to the prediction of how patients may respond to conventional antifungal therapy, allowing the discrimination of patients that require enhanced surveillance for fungal disease and/or alternative antifungal therapies.

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