

Immunogenetic profiling to predict risk of invasive fungal diseases: where are we now?

Journal:	<i>Immunological Investigations</i>
Manuscript ID:	Draft
Manuscript Type:	Reviews
Date Submitted by the Author:	n/a
Complete List of Authors:	Cunha, Cristina; University of Perugia, Department of Experimental Medicine and Biochemical Sciences Aversa, Franco; University of Perugia, Department of Clinical and Experimental Medicine Bistoni, Giovanni; University of Perugia, Department of Experimental Medicine and Biochemical Sciences Casagrande, Andrea; University of Perugia, Department of Experimental Medicine and Biochemical Sciences Rodrigues, Fernando; University of Minho, School of Health Sciences Romani, Luigina; University of Perugia, Department of Experimental Medicine and Biochemical Sciences Carvalho, Agostinho; University of Perugia, Dep. of Experimental Medicine and Biochemical Sciences
Keywords:	Fungal disease, Genetic susceptibility, Pattern recognition receptors, Inflammation

SCHOLARONE™
Manuscripts

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

For Peer Review Only

Immunogenetic profiling to predict risk of invasive fungal diseases: where are we now?

Running head: Immunogenetics of invasive fungal diseases

Cristina Cunha¹, Franco Aversa², Giovanni Bistoni¹, Andrea Casagrande¹, Fernando Rodrigues³, Luigina Romani¹ and Agostinho Carvalho^{1,3}

¹ Microbiology Section, Department of Experimental Medicine and Biochemical Sciences, University of Perugia, Perugia, Italy

² Division of Hematology and Clinical Immunology, Department of Clinical and Experimental Medicine, University of Perugia, Perugia, Italy

³ Life and Health Sciences Research Institute (ICVS), School of Health Sciences, University of Minho, Braga, Portugal

Correspondence: Agostinho Carvalho, Microbiology Section, Department of Experimental Medicine and Biochemical Sciences, University of Perugia, Via del Giochetto, 06126 Perugia, Italy.

Ph and Fax: +39 075 5857498. E-mail: acarvalho2008@gmail.com

Abstract

Invasive fungal diseases remain nowadays life-threatening conditions affecting multiple clinical settings. The onset of these diseases is dependent on numerous factors, of which the “immunocompromised” phenotype of the patients is the more often acknowledged. However, and despite comparable immune dysfunction, not all patients are ultimately susceptible to disease, suggesting that additional risk factors, likely of genetic nature, may also be important. In the last years, genetic variants in several immune-related genes have also been proposed as major determinants of the susceptibility pattern of high-risk patients to invasive fungal diseases. Altogether, these findings highlighted the crucial significance of the individual genetic make-up in defining susceptibility to infection, providing a compelling rationale for the introduction of the immunogenetic profile as a risk prediction measure that may ultimately help to guide clinicians in the use of prophylaxis and preemptive fungal therapy in high-risk patients.

Introduction

Fungal diseases are epidemiologically characteristic of high-risk groups. These include patients with hematological malignancies, prolonged and severe neutropenia, undergoing corticosteroid therapy or submitted to solid organ or stem cell transplantation, among others. However, besides the relative risk conferred by the “immunocompromised” phenotype as a result of the underlying condition or treatment scheme, certain individuals with specific, genetically-determined, immune defects are also more vulnerable to infection.

Over the last decades, considerable advances have been made in understanding the molecular bases determining disparate susceptibilities to infectious diseases in humans. Monogenetic defects underlying primary immunodeficiencies (PIDs) have been classically associated with predisposition to a restricted spectrum of fungal pathogens causing clinically defined manifestations. For example, the hyper-IgE syndrome (HIES) or the autoimmune polyendocrinopathy candidiasis ectodermal dystrophy (APECED) syndrome are usually correlated with susceptibility to superficial fungal infections, most remarkably chronic mucocutaneous candidiasis (CMC). On the other hand, chronic granulomatous disease (CGD), an inherited disorder of the phagocyte nicotinamide adenine dinucleotide phosphate (NADPH) oxidase complex that impairs the production of reactive oxygen species, predisposes to several recurrent life-threatening infections, of which invasive aspergillosis (IA) is the most distinctive fungal disease.

In addition to PIDs, other factors modifying risk of fungal disease are often genetic polymorphisms. These have a substantial allele prevalence in the general population but, under select contexts of immunocompromise typical of many clinical settings, may translate into a further severity of the immunological dysfunction, ultimately rendering these individuals more prone to disease. In this article, we review the contribution of genetic polymorphisms of the immune system to susceptibility to invasive fungal diseases and discuss how this knowledge may be used for the design of individually tailored approaches to be integrated in clinical practice.

Genetic defects of PRRs and susceptibility to invasive fungal diseases

Invasive fungal diseases are usually devastating conditions associated with high mortality rates and that affect a broad range of patients with apparently disparate underlying risk factors (e.g. allogeneic stem cell transplant recipients). Given the need to predict risk for disease in these patients and design effective preventive strategies, a relatively large number of studies have investigated the association between genetic variants of pattern recognition receptors (PRRs) or inflammatory mediators and risk of invasive fungal diseases (Carvalho *et al.*, 2010b) (Figure 1).

Toll-like receptors (TLRs)

TLRs participate in the recognition of microbial structures and in the initiation of inflammatory and antimicrobial host defenses. They are expressed in several cell types including, but not exclusively, monocytes, macrophages, dendritic cells and neutrophils, and can be predominantly expressed on the cell surface (TLR1, -2, -4, -5 and -6) or retained intracellularly in endosomes (TLR3, -7, -8 and -9). Recent evidence also attested a major contribution of TLRs present at epithelial surfaces to antimicrobial defense and immunosurveillance (Weindl *et al.*, 2007; de Luca *et al.*, 2010). Distinct TLR ligands are able to mediate disparate effector responses through the same receptor, a phenomenon in part explained by the selective usage of adapter molecules such as myeloid differentiation factor 88 (MyD88) and Toll/IL-1 receptor (TIR) domain-containing adapter inducing interferon- β (TRIF). Receptor engagement eventually culminates in the activation of transcription factors such as nuclear factor κ B (NF- κ B) and members of the interferon regulatory factor (IRF) family, which successively induce gene expression and production of various cytokines, chemokines and molecules required for antigen presentation or costimulation.

The early finding that Toll-deficient *Drosophila* were unable to mount effective antifungal responses and were highly susceptible to infection by *Aspergillus fumigatus* highlighted a similar participation of mammalian TLRs in antifungal immunity. In fact, TLR2, -4 and -9 function has

1
2
3 been categorically demonstrated to contribute to host responses against fungi both in mice and
4
5 in humans (Cunha *et al.*, 2010c). Given its crucial role in the immune response to fungi, TLR4 has
6
7 been regarded as one major potential repository of genetic variability contributing to
8
9 susceptibility to fungal diseases. For this reason, most studies performed to date have focused
10
11 on the highly polymorphic *TLR4* gene, in which two nonsynonymous polymorphisms – D299G
12
13 and T399I – have been linked with blunted responses to inhaled lipopolysaccharide (Arbour *et*
14
15 *al.*, 2000).
16
17

18
19 A donor haplotype in *TLR4* consisting of the above-mentioned polymorphisms was
20
21 disclosed as an independent predictive factor for IA among unrelated hematopoietic stem cell
22
23 transplant (HSCT) recipients (Bochud *et al.*, 2008). Although alternative explanations for this
24
25 association have been proposed, namely the possible interaction of TLR4 with cytomegalovirus
26
27 (CMV) (Cervera *et al.*, 2009) or antifungal drugs (Levitz *et al.*, 2009), the D299G and T399I
28
29 polymorphisms have been linked with susceptibility to pulmonary aspergillosis in non-
30
31 transplanted patients, who are not susceptible to CMV disease and usually do not receive
32
33 antifungal prophylaxis (Carvalho *et al.*, 2008). In addition, we have reported an association of
34
35 the *TLR4* haplotype with fungal colonization following T-cell-depleted transplantation, although
36
37 without increased risk for invasive disease (Carvalho *et al.*, 2009). A previous study had also
38
39 failed to associate *TLR4* polymorphisms with IA in HSCT recipients, despite evidence pointing to
40
41 a role of genetic variants in *TLR1* and *TLR6* (Kesh *et al.*, 2005). These discrepancies between
42
43 studies further stress that the contribution of genetic variants to IA may depend on several
44
45 factors, such as the type of transplant and associated demographic and clinical co-variables.
46
47 Uncovering the biological implications of *TLR4* polymorphisms in the immune response to
48
49 *Aspergillus* would ultimately strengthen the clinical repercussions of these findings. It is worth
50
51 mentioning that polymorphisms in *TLR2* and *TLR9* were not associated with IA (Bochud *et al.*,
52
53 2008; Carvalho *et al.*, 2009), although a common promoter polymorphism in *TLR9* (-1237T>C)
54
55 was shown to predispose to allergic bronchopulmonary aspergillosis (Carvalho *et al.*, 2008).
56
57
58
59
60

1
2
3 However, it also holds true that the absence of association should always be interpreted in the
4 scope of the limited power of the existing studies.
5
6

7 The *TLR4* haplotype had also been suggested to contribute to a higher risk of *Candida*
8 bloodstream infection (Van der Graaf *et al.*, 2006). However, this underpowered report has been
9 recently challenged by a study from the same group in a large cohort of patients with invasive
10 candidiasis (IC), which excluded any role of these genetic variants in susceptibility to this
11 disease (Plantinga *et al.*, 2009a). Instead, polymorphisms in *TLR1* were shown to be strong
12 predictive markers for IC and functional assays demonstrated that the increased risk was
13 associated with impaired production of cytokines such as IL-1 β , IL-6 and IL-8. Although this
14 provides proof of concept that TLR1 plays a major role in the pathogenesis of IC, it is of note that
15 this study dealt only with nonsynonymous polymorphisms and thus, does not exclude additional
16 genetic variability potentially impacting susceptibility to this disease.
17
18
19
20
21
22
23
24
25
26
27
28

29 In addition, and despite no studies have revealed an association between genetic
30 variants in *TLR2* and candidiasis, the nonsynonymous polymorphism R753Q was shown to
31 modulate cytokine release during *Candida* sepsis in intensive care unit patients (Woehrle *et al.*,
32 2008). The extent to which the deregulated cytokine production contributes to susceptibility to
33 candidiasis is however still not clear.
34
35
36
37
38
39
40
41
42

43 *C-type lectin receptors (CLRs)*

44 Even though the signaling pathways elicited by TLRs are crucial for the control of fungal
45 infection (Bellocchio *et al.*, 2004), a pivotal role for dectin-1 as the prototype of innate non-TLR
46 signaling pathway for antifungal sensing has been highlighted (Brown, 2006). Dectin-1 is a CLR
47 that specifically recognizes the cell wall carbohydrate β -(1,3)-glucan of many fungi and mediates
48 cell activation, cytokine production and a variety of antifungal responses either through the
49 spleen tyrosine kinase Syk/cytoplasmic caspase recruiting domain 9 (CARD9) (LeibundGut-
50 Landmann *et al.*, 2007) or Raf-1 (Gringhuis *et al.*, 2009) pathways.
51
52
53
54
55
56
57
58
59
60

1
2
3 Polymorphisms in the gene encoding for dectin-1 have also been recently addressed as
4 potential predictive factors for the incidence of fungal diseases. In particular, the early stop
5 codon polymorphism Y238X has been found to associate with recurrent mucocutaneous fungal
6 infections (Ferwerda *et al.*, 2009) and to contribute to *Candida* colonization after HSCT
7 (Plantinga *et al.*, 2009b). This susceptibility phenotype was correlated with the generation of a
8 truncated dectin-1 protein unable to target the membrane, thereby restraining β -glucan binding
9 by monocytes and resulting in defective cytokine production upon receptor engagement, in
10 particular IL-17 (Ferwerda *et al.*, 2009; Plantinga *et al.*, 2009b). Interestingly, a similar
11 susceptibility pattern to mucocutaneous infections related to a deficit in IL-17 production was
12 also found in a family carrying loss-of-function mutations in *CARD9* (Glocker *et al.*, 2009),
13 suggesting that disruption of dectin-1 signaling and concomitant failure to produce IL-17 in
14 response to *Candida* critically impairs mucosal antifungal defense. Accordingly, the clinical
15 manifestation of CMC was also recently found to rely on genetic defects in *IL17F* and *IL17RA*
16 (Puel *et al.*, 2011).
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33

34 Work from our group has also demonstrated that dectin-1 deficiency contributed as well
35 to an increased susceptibility to IA following HSCT, an association which relied on either
36 recipient or donor genetic make-ups and to be synergistically increased in polymorphic
37 recipient/donor pairs (Cunha *et al.*, 2010a). Another study has instead proposed a limited
38 influence of the Y238X polymorphism on susceptibility to IA, in particular in non-HSCT patients
39 (Chai *et al.*, 2011). This study however failed to address the likely confounding effects of
40 population stratification on their conclusions given the enrollment of admixed heterogeneous
41 patient populations. Larger, well-designed studies, preferably performed on consecutive
42 patients, are ultimately required to clarify the role of the Y238X polymorphism in both HSCT and
43 non-HSCT patients at risk of IA.
44
45
46
47
48
49
50
51
52
53
54
55

56 Although dectin-1 has been regarded as one major innate receptor leading to T helper 17
57 (Th17) activation in response to *Aspergillus* (Werner *et al.*, 2009), production of IFN- γ and IL-10
58 by dectin-1-deficient peripheral blood mononuclear cells, more than IL-17, was found to be
59
60

1
2
3 impaired (Cunha *et al.*, 2010a). Interestingly, polymorphic macrophages displayed instead
4
5 adequate responses to the fungus (Chai *et al.*, 2011). This suggests that pulmonary macrophages,
6
7 in view of their role as primary line of defense, retain their ability to properly respond to
8
9 *Aspergillus* ligands even in the absence of functional dectin-1, likely due to the inherent
10
11 redundancy of the several PRRs (e.g. mannose receptor, TLR2, TLR4, etc.). Moreover, the
12
13 contribution of recipient dectin-1 deficiency to the high risk of infection also reflects the crucial
14
15 role of nonhematopoietic dectin-1 in the induction of immune protection to the fungus,
16
17 highlighting the overlapping mechanisms of immune resistance and tolerance that are
18
19 conditioned by the hematopoietic/nonhematopoietic compartmentalization.
20
21
22
23
24

25 *Other PRRs*

26
27 The collectin subfamily of lectins includes members such as mannose-binding lectin
28
29 (MBL), pentraxin 3 (PTX3) and the lung surfactant proteins (SPs). Collectins are able to bind to
30
31 fungi and activate the complement system leading to their opsonization or direct killing. Among
32
33 this class of PRRs, MBL is the most extensively dissected member from a genetic point of view. A
34
35 combination of structural and promoter polymorphisms is known to exist in the *MBL2* gene and
36
37 each may define the levels of functional protein (Garred, 2008). For this reason, since it was first
38
39 reported, MBL deficiency has been consistently associated with increased susceptibility to
40
41 infections, particularly when adaptive immunity is impaired. Accordingly, reduced levels of MBL
42
43 have been recently correlated with the incidence of acute IA in non-HSCT immunocompromised
44
45 patients, despite no causal nature was attributed to MBL polymorphisms (Lambourne *et al.*,
46
47 2009). Nevertheless, donor *MBL2*^{low} genotypes (O/O or LXA/O) have been identified as
48
49 important predictive factors of increased incidence of invasive fungal diseases after HSCT
50
51 (Granell *et al.*, 2006). Similarly, the nonsynonymous polymorphism D105G in recipient MBL-
52
53 associated serine protease 2 gene (*MASP2*), known to hinder MBL function, was also linked with
54
55 invasive fungal disease after HSCT (Granell *et al.*, 2006). However, the later finding deserves
56
57 additional validation owing to the low number of recipients with the *MASP2* variant.
58
59
60

1
2
3 In addition to MBL, genetic mapping analysis of the survival data of an
4 immunocompromised mice model has also allowed the identification of plasminogen, a
5 regulatory molecule that binds to *Aspergillus*, as a suitable candidate gene for aspergillosis
6 susceptibility (Zaas *et al.*, 2008). The clinical translation of these findings acknowledged the
7 nonsynonymous polymorphism D472N in the human gene encoding for plasminogen to
8 influence the risk of developing IA in HSCT recipients, particularly late after transplantation.
9 Besides shedding light into the role of the fibrinolytic system in the pathogenesis of IA, this
10 computational approach identified a novel and biologically plausible candidate gene for
11 susceptibility to IA, therefore validating its future use in the identification of less obvious fungal
12 disease-related genes.
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27

28 *Cytokines/chemokines*

29 Cytokines mediate the inflammatory and adaptive immune responses to pathogens. Whereas
30 inflammation may serve to limit infection, when heightened, it may significantly contribute to
31 pathogenicity, as documented by the elevated incidence of severe fungal infections in patients
32 with immune reconstitution syndrome (Singh and Perfect, 2007). It is not surprising therefore
33 that patients with inborn errors in the IL-12/IFN- γ axis, unable to mount proper inflammatory
34 responses, are highly susceptible to disseminated fungal diseases such as
35 paracoccidioidomycosis (PCM) (Moraes-Vasconcelos *et al.*, 2005) and histoplasmosis (Zerbe and
36 Holland, 2005).
37
38
39
40
41
42
43
44
45
46
47

48 Genetic variants in cytokine genes have been considered crucial factors determining
49 vulnerability to fungal infections, particularly aspergillosis. In this regard, IL-10 has been
50 considered one major candidate for genetic studies as its production has been suggested to be
51 largely genetically-determined, in part due to the promoter polymorphism -1082G>A. Indeed,
52 patient *IL10^{low}* genotypes were shown to confer protection from IA in both HSCT (Seo *et al.*,
53 2005) and non-HSCT (Sainz *et al.*, 2007a) patients. Despite the fact that no actual correlation
54 with serum levels was established in these studies, the validity of these associations is fairly
55
56
57
58
59
60

1
2
3 reinforced by the finding that *IL10^{high}* genotypes were instead linked with increased colonization
4 with *Aspergillus* and allergic disease in patients with cystic fibrosis (Brouard *et al.*, 2005).
5
6 Similarly, increased production of IL-10 resulting from *IL10^{high}* genotypes has also been shown
7
8 to contribute to the development of chronic fungal infections such as PCM (Bozzi *et al.*, 2006).
9
10 Hence, and regardless of the lack of functional data definitely correlating *IL10* polymorphisms
11
12 with impaired immune responses to *Aspergillus*, the consistency of the associations based on
13
14 genotype-defined IL-10 levels reflects the likely role of these polymorphisms in determining
15
16 resistance versus susceptibility to aspergillosis.
17
18
19

20
21 A small number of other studies have addressed the role of polymorphisms in additional
22
23 cytokines and/or their receptors as predisposing factors to IA. For example, and despite analysis
24
25 of individual locus failed to associate variants in the genes encoding for IL-1 α , IL-1 β and IL-1R
26
27 antagonist (IL-1Ra) with IA, a polymorphism haplotype in this gene cluster (VNTR2/-889C/-
28
29 511T) was nevertheless strongly associated with susceptibility to this disease in hematological
30
31 patients (Sainz *et al.*, 2008). Work from the same group has also demonstrated a crucial
32
33 contribution of polymorphisms in the TNF receptor 1 (*TNFR1*) and 2 (*TNFR2*) to an increased
34
35 susceptibility to IA in non-HSCT patients (Sainz *et al.*, 2007b; Sainz *et al.*, 2010). In this case, and
36
37 particularly regarding TNFR1, the observed susceptibility to IA was correlated with defective
38
39 receptor expression, suggesting a crucial contribution of TNF- α signaling in the immune
40
41 response to *Aspergillus*, at least in this particular setting of patients.
42
43
44

45
46 In addition, we have found that the functional polymorphism R381Q affecting the
47
48 receptor for IL-23 (IL-23R) instead conferred a protective effect regarding IA and correlated
49
50 with improved survival of HSCT recipients (Carvalho *et al.*, 2010a). Functionally, this specific
51
52 polymorphism has been demonstrated to promote a deficient activation of IL-23-driven Th17
53
54 responses (Cunha *et al.*, 2010b). Interestingly, whereas committed effector Th17 cells derived
55
56 from subjects harboring R381Q stimulated with IL-23 had an impaired production of IL-17,
57
58 highly purified naïve T-cells did not, suggesting that the consequences of the polymorphism may
59
60 be exclusively reflected in the function of differentiated Th17 cells.

1
2
3 Recently, data has also pointed to the relevance of genetic variants in chemokine genes in
4 host susceptibility to fungal diseases. A large-scale screening of polymorphisms led to the
5 finding of a haplotype in CXC chemokine ligand 10 (*CXCL10*) resulting in increased susceptibility
6 to IA in HSCT recipients (Mezger *et al.*, 2008). Mechanistically, immature wild-type dendritic
7 cells exposed to *A. fumigatus* showed a markedly increase in *CXCL10* expression, in contrast to
8 those harboring the risk haplotype. In this regard, it is also interesting to note that patients who
9 survived IA had significantly higher *CXCL10* levels in comparison to healthy controls.
10
11

12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
Altogether, and despite the need for replication studies in independent cohorts of patients, these findings point to the importance of maintaining a finely orchestrated balance between pro- and anti-inflammatory signals, fundamental for the immune system to effectively attack and eliminate pathogenic fungi, suggesting that the disruption of this equilibrium may ultimately underlie an increased predisposition to IA.

Immunogenetics of invasive fungal diseases: what does the future hold?

The dissection of the genetic bases underlying susceptibility to infectious diseases inspires a very active, yet complex, field of research. As the awareness of the individual genetic make-up may hold the key to expose unsuspected risk factors for these diseases, the current understanding of the immunological network involved in the immune response to pathogens needs to be addressed also from a genetic point of view. Given that the modulation of host immune responses has been regarded as a potential therapeutic target for the eradication of fungal diseases, knowing each individual's immunogenetic profile, together with the application of promising therapeutic strategies such as siRNA delivery (Bonifazi *et al.*, 2010), could prove critical for the design of novel, effective fungal vaccines capable of targeting and exerting control over the outcome of specific signalling pathways.

Although many of the studies published so far still lack replication, a step forward in this field would be provided by large-scale translational and clinical studies validating the data obtained from the genetic analyses. This would support the use of genetic screening of at-risk

1
2
3 patients and would ultimately allow the individualization of prophylaxis and treatments by
4
5 means of targeted and patient-tailored approaches likely improving the management and
6
7 outcome of these severe, often fatal diseases.
8
9

10 11 **Acknowledgements**

12 This work was supported by the Specific Targeted Research Projects “ALLFUN” (FP7-HEALTH-
13
14 2010-single-stage, contract number 260338) and “SYBARIS” (FP7-HEALTH-2009-single-stage
15
16 contract number 037899) (FP7), and by the Fondazione per la Ricerca sulla Fibrosi Cistica
17
18 (Project number FFC#21/2010). Cristina Cunha and Agostinho Carvalho were financially
19
20 supported by fellowships from Fundação para a Ciência e Tecnologia, Portugal (contracts
21
22 SFRH/BD/65962/2009 and SFRH/BPD/46292/2008, respectively).
23
24
25
26
27
28

29 **Declaration of interest**

30 The authors report no conflicts of interest. The authors alone are responsible for the content and
31
32 writing of the paper.
33
34
35
36
37

38 **Figure legends**

39
40 **Figure 1.** Genetic polymorphisms of pattern recognition receptors (PRRs) and inflammatory
41
42 mediators associated with susceptibility to invasive fungal diseases.
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

References

- Arbour, N.C., Lorenz, E., Schutte, B.C., Zabner, J., Kline, J.N., Jones, M., Frees, K., Watt, J.L., and Schwartz, D.A. (2000). TLR4 mutations are associated with endotoxin hyporesponsiveness in humans. *Nat Genet* 25, 187-191.
- Bellocchio, S., Montagnoli, C., Bozza, S., Gaziano, R., Rossi, G., Mambula, S.S., Vecchi, A., Mantovani, A., Levitz, S.M., and Romani, L. (2004). The contribution of the Toll-like/IL-1 receptor superfamily to innate and adaptive immunity to fungal pathogens in vivo. *J Immunol* 172, 3059-3069.
- Bochud, P.Y., Chien, J.W., Marr, K.A., Leisenring, W.M., Upton, A., Janer, M., Rodrigues, S.D., Li, S., Hansen, J.A., Zhao, L.P., Aderem, A., and Boeckh, M. (2008). Toll-like receptor 4 polymorphisms and aspergillosis in stem-cell transplantation. *N Engl J Med* 359, 1766-1777.
- Bonifazi, P., D'Angelo, C., Zagarella, S., Zelante, T., Bozza, S., De Luca, A., Giovannini, G., Moretti, S., Iannitti, R.G., Fallarino, F., Carvalho, A., Cunha, C., Bistoni, F., and Romani, L. (2010). Intranasally delivered siRNA targeting PI3K/Akt/mTOR inflammatory pathways protects from aspergillosis. *Mucosal Immunol* 3, 193-205.
- Bozzi, A., Pereira, P.P., Reis, B.S., Goulart, M.I., Pereira, M.C., Pedroso, E.P., Leite, M.F., and Goes, A.M. (2006). Interleukin-10 and tumor necrosis factor-alpha single nucleotide gene polymorphism frequency in paracoccidioidomycosis. *Hum Immunol* 67, 931-939.
- Brouard, J., Knauer, N., Boelle, P.Y., Corvol, H., Henrion-Caude, A., Flamant, C., Bremont, F., Delaisi, B., Duhamel, J.F., Marguet, C., Roussey, M., Miesch, M.C., Chadelat, K., Boule, M., Fauroux, B., Ratjen, F., Grasemann, H., and Clement, A. (2005). Influence of interleukin-10 on *Aspergillus fumigatus* infection in patients with cystic fibrosis. *J Infect Dis* 191, 1988-1991.
- Brown, G.D. (2006). Dectin-1: a signalling non-TLR pattern-recognition receptor. *Nat Rev Immunol* 6, 33-43.
- Carvalho, A., Cunha, C., Carotti, A., Aloisi, T., Guarrera, O., Di Ianni, M., Falzetti, F., Bistoni, F., Aversa, F., Pitzurra, L., Rodrigues, F., and Romani, L. (2009). Polymorphisms in Toll-like receptor

1
2
3 genes and susceptibility to infections in allogeneic stem cell transplantation. *Exp Hematol* 37,
4 1022-1029.

5
6
7 Carvalho, A., Cunha, C., Di Ianni, M., Pitzurra, L., Aloisi, T., Falzetti, F., Carotti, A., Bistoni, F.,
8 Aversa, F., and Romani, L. (2010a). Prognostic significance of genetic variants in the IL-23/Th17
9 pathway for the outcome of T cell-depleted allogeneic stem cell transplantation. *Bone Marrow*
10 *Transplant* 45, 1645-1652.

11
12
13 Carvalho, A., Cunha, C., Pasqualotto, A.C., Pitzurra, L., Denning, D.W., and Romani, L. (2010b).
14 Genetic variability of innate immunity impacts human susceptibility to fungal diseases. *Int J*
15 *Infect Dis* 14, e460-468.

16
17
18 Carvalho, A., Pasqualotto, A.C., Pitzurra, L., Romani, L., Denning, D.W., and Rodrigues, F. (2008).
19 Polymorphisms in toll-like receptor genes and susceptibility to pulmonary aspergillosis. *J Infect*
20 *Dis* 197, 618-621.

21
22
23 Cervera, C., Moreno, A., and Lozano, F. (2009). Toll-like receptor 4 polymorphisms and
24 aspergillosis. *N Engl J Med* 360, 634-635; author reply 635-636.

25
26
27 Chai, L.Y., de Boer, M.G., van der Velden, W.J., Plantinga, T.S., van Spruiel, A.B., Jacobs, C., Halkes,
28 C.J., Vonk, A.G., Blijlevens, N.M., van Dissel, J.T., Donnelly, P.J., Kullberg, B.J., Maertens, J., and
29 Netea, M.G. (2011). The Y238X Stop Codon Polymorphism in the Human {beta}-Glucan Receptor
30 Dectin-1 and Susceptibility to Invasive Aspergillosis. *J Infect Dis* 203, 736-743.

31
32
33 Cunha, C., Di Ianni, M., Bozza, S., Giovannini, G., Zagarella, S., Zelante, T., D'Angelo, C., Pierini, A.,
34 Pitzurra, L., Falzetti, F., Carotti, A., Perruccio, K., Latge, J.P., Rodrigues, F., Velardi, A., Aversa, F.,
35 Romani, L., and Carvalho, A. (2010a). Dectin-1 Y238X polymorphism associates with
36 susceptibility to invasive aspergillosis in hematopoietic transplantation through impairment of
37 both recipient- and donor-dependent mechanisms of antifungal immunity. *Blood* 116, 5394-
38 5402.

39
40
41 Cunha, C., Rodrigues, F., Zelante, T., Aversa, F., Romani, L., and Carvalho, A. (2010b). Genetic
42 susceptibility to aspergillosis in allogeneic stem-cell transplantation. *Med Mycol*.

1
2
3 Cunha, C., Romani, L., and Carvalho, A. (2010c). Cracking the Toll-like receptor code in fungal
4 infections. *Expert Rev Anti Infect Ther* 8, 1121-1137.

5
6
7 de Luca, A., Bozza, S., Zelante, T., Zagarella, S., D'Angelo, C., Perruccio, K., Vacca, C., Carvalho, A.,
8 Cunha, C., Aversa, F., and Romani, L. (2010). Non-hematopoietic cells contribute to protective
9 tolerance to *Aspergillus fumigatus* via a TRIF pathway converging on IDO. *Cell Mol Immunol* 7,
10 459-470.

11
12
13
14
15
16 Ferwerda, B., Ferwerda, G., Plantinga, T.S., Willment, J.A., van Sriel, A.B., Venselaar, H., Elbers,
17 C.C., Johnson, M.D., Cambi, A., Huysamen, C., Jacobs, L., Jansen, T., Verheijen, K., Masthoff, L.,
18 Morre, S.A., Vriend, G., Williams, D.L., Perfect, J.R., Joosten, L.A., Wijmenga, C., van der Meer, J.W.,
19 Adema, G.J., Kullberg, B.J., Brown, G.D., and Netea, M.G. (2009). Human dectin-1 deficiency and
20 mucocutaneous fungal infections. *N Engl J Med* 361, 1760-1767.

21
22
23
24
25
26
27 Garred, P. (2008). Mannose-binding lectin genetics: from A to Z. *Biochem Soc Trans* 36, 1461-
28 1466.

29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
Glocker, E.O., Hennigs, A., Nabavi, M., Schaffer, A.A., Woellner, C., Salzer, U., Pfeifer, D., Veelken, H.,
Warnatz, K., Tahami, F., Jamal, S., Manguiat, A., Rezaei, N., Amirzargar, A.A., Plebani, A.,
Hanneschlager, N., Gross, O., Ruland, J., and Grimbacher, B. (2009). A homozygous CARD9
mutation in a family with susceptibility to fungal infections. *N Engl J Med* 361, 1727-1735.

Granell, M., Urbano-Ispizua, A., Suarez, B., Rovira, M., Fernandez-Aviles, F., Martinez, C., Ortega,
M., Uriburu, C., Gaya, A., Roncero, J.M., Navarro, A., Carreras, E., Mensa, J., Vives, J., Rozman, C.,
Montserrat, E., and Lozano, F. (2006). Mannan-binding lectin pathway deficiencies and invasive
fungal infections following allogeneic stem cell transplantation. *Exp Hematol* 34, 1435-1441.

Gringhuis, S.I., den Dunnen, J., Litjens, M., van der Vlist, M., Wevers, B., Bruijns, S.C., and
Geijtenbeek, T.B. (2009). Dectin-1 directs T helper cell differentiation by controlling
noncanonical NF-kappaB activation through Raf-1 and Syk. *Nat Immunol* 10, 203-213.

Kesh, S., Mensah, N.Y., Peterlongo, P., Jaffe, D., Hsu, K., M, V.D.B., O'Reilly, R., Pamer, E., Satagopan,
J., and Papanicolaou, G.A. (2005). TLR1 and TLR6 polymorphisms are associated with

1
2
3 susceptibility to invasive aspergillosis after allogeneic stem cell transplantation. *Ann N Y Acad*
4
5 *Sci* 1062, 95-103.

6
7 Lambourne, J., Agranoff, D., Herbrecht, R., Troke, P.F., Buchbinder, A., Willis, F., Letscher-Bru, V.,
8
9 Agrawal, S., Doffman, S., Johnson, E., White, P.L., Barnes, R.A., Griffin, G., Lindsay, J.A., and
10
11 Harrison, T.S. (2009). Association of mannose-binding lectin deficiency with acute invasive
12
13 aspergillosis in immunocompromised patients. *Clin Infect Dis* 49, 1486-1491.

14
15 LeibundGut-Landmann, S., Gross, O., Robinson, M.J., Osorio, F., Slack, E.C., Tsoni, S.V.,
16
17 Schweighoffer, E., Tybulewicz, V., Brown, G.D., Ruland, J., and Reis e Sousa, C. (2007). Syk- and
18
19 CARD9-dependent coupling of innate immunity to the induction of T helper cells that produce
20
21 interleukin 17. *Nat Immunol* 8, 630-638.

22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
Levitz, S.M., Shoham, S., and Cleary, J.D. (2009). Toll-like receptor 4 polymorphisms and
aspergillosis. *N Engl J Med* 360, 634; author reply 635-636.

Mezger, M., Steffens, M., Beyer, M., Manger, C., Eberle, J., Toliat, M.R., Wienker, T.F., Ljungman, P.,
Hebart, H., Dornbusch, H.J., Einsele, H., and Loeffler, J. (2008). Polymorphisms in the chemokine
(C-X-C motif) ligand 10 are associated with invasive aspergillosis after allogeneic stem-cell
transplantation and influence CXCL10 expression in monocyte-derived dendritic cells. *Blood*
111, 534-536.

Moraes-Vasconcelos, D., Grumach, A.S., Yamaguti, A., Andrade, M.E., Fieschi, C., de Beaucoudrey,
L., Casanova, J.L., and Duarte, A.J. (2005). Paracoccidioides brasiliensis disseminated disease in a
patient with inherited deficiency in the beta1 subunit of the interleukin (IL)-12/IL-23 receptor.
Clin Infect Dis 41, e31-37.

Plantinga, T.S., Johnsson, M., Scott, B., van de Vosse, E., Velez, D., van der Meer, J.W.M., van Dissel,
J., Perfect, J., Kullberg, B.J., and Netea, M.G. (2009a). Toll like receptor 1 polymorphisms and
susceptibility to invasive candidiasis. *Sepsis* 13 (Suppl 4), P28.

Plantinga, T.S., van der Velden, W.J., Ferwerda, B., van Spriel, A.B., Adema, G., Feuth, T., Donnelly,
J.P., Brown, G.D., Kullberg, B.J., Blijlevens, N.M., and Netea, M.G. (2009b). Early stop

1
2
3 polymorphism in human DECTIN-1 is associated with increased candida colonization in
4 hematopoietic stem cell transplant recipients. *Clin Infect Dis* 49, 724-732.

5
6
7 Puel, A., Cypowyj, S., Bustamante, J., Wright, J.F., Liu, L., Lim, H.K., Migaud, M., Israel, L., Chrabieh,
8 M., Audry, M., Gumbleton, M., Toulon, A., Bodemer, C., El-Baghdadi, J., Whitters, M., Paradis, T.,
9
10 Brooks, J., Collins, M., Wolfman, N.M., Al-Muhsen, S., Galicchio, M., Abel, L., Picard, C., and
11
12 Casanova, J.L. (2011). Chronic Mucocutaneous Candidiasis in Humans with Inborn Errors of
13
14 Interleukin-17 Immunity. *Science*.

15
16
17 Sainz, J., Hassan, L., Perez, E., Romero, A., Moratalla, A., Lopez-Fernandez, E., Oyonarte, S., and
18
19 Jurado, M. (2007a). Interleukin-10 promoter polymorphism as risk factor to develop invasive
20
21 pulmonary aspergillosis. *Immunol Lett* 109, 76-82.

22
23
24 Sainz, J., Perez, E., Gomez-Lopera, S., and Jurado, M. (2008). IL1 gene cluster polymorphisms and
25
26 its haplotypes may predict the risk to develop invasive pulmonary aspergillosis and modulate C-
27
28 reactive protein level. *J Clin Immunol* 28, 473-485.

29
30
31 Sainz, J., Perez, E., Hassan, L., Moratalla, A., Romero, A., Collado, M.D., and Jurado, M. (2007b).
32
33 Variable number of tandem repeats of TNF receptor type 2 promoter as genetic biomarker of
34
35 susceptibility to develop invasive pulmonary aspergillosis. *Hum Immunol* 68, 41-50.

36
37
38 Sainz, J., Salas-Alvarado, I., Lopez-Fernandez, E., Olmedo, C., Comino, A., Garcia, F., Blanco, A.,
39
40 Gomez-Lopera, S., Oyonarte, S., Bueno, P., and Jurado, M. (2010). TNFR1 mRNA expression level
41
42 and TNFR1 gene polymorphisms are predictive markers for susceptibility to develop invasive
43
44 pulmonary aspergillosis. *Int J Immunopathol Pharmacol* 23, 423-436.

45
46
47 Seo, K.W., Kim, D.H., Sohn, S.K., Lee, N.Y., Chang, H.H., Kim, S.W., Jeon, S.B., Baek, J.H., Kim, J.G.,
48
49 Suh, J.S., and Lee, K.B. (2005). Protective role of interleukin-10 promoter gene polymorphism in
50
51 the pathogenesis of invasive pulmonary aspergillosis after allogeneic stem cell transplantation.
52
53 *Bone Marrow Transplant* 36, 1089-1095.

54
55
56 Singh, N., and Perfect, J.R. (2007). Immune reconstitution syndrome associated with
57
58 opportunistic mycoses. *Lancet Infect Dis* 7, 395-401.

1
2
3 Van der Graaf, C.A., Netea, M.G., Morre, S.A., Den Heijer, M., Verweij, P.E., Van der Meer, J.W., and
4
5 Kullberg, B.J. (2006). Toll-like receptor 4 Asp299Gly/Thr399Ile polymorphisms are a risk factor
6
7 for *Candida* bloodstream infection. *Eur Cytokine Netw* 17, 29-34.

8
9
10 Weindl, G., Naglik, J.R., Kaesler, S., Biedermann, T., Hube, B., Korting, H.C., and Schaller, M. (2007).
11
12 Human epithelial cells establish direct antifungal defense through TLR4-mediated signaling. *J*
13
14 *Clin Invest* 117, 3664-3672.

15
16 Werner, J.L., Metz, A.E., Horn, D., Schoeb, T.R., Hewitt, M.M., Schwiebert, L.M., Faro-Trindade, I.,
17
18 Brown, G.D., and Steele, C. (2009). Requisite role for the dectin-1 beta-glucan receptor in
19
20 pulmonary defense against *Aspergillus fumigatus*. *J Immunol* 182, 4938-4946.

21
22
23 Woehrle, T., Du, W., Goetz, A., Hsu, H.Y., Joos, T.O., Weiss, M., Bauer, U., Brueckner, U.B., and
24
25 Marion Schneider, E. (2008). Pathogen specific cytokine release reveals an effect of TLR2
26
27 Arg753Gln during *Candida* sepsis in humans. *Cytokine* 41, 322-329.

28
29
30 Zaas, A.K., Liao, G., Chien, J.W., Weinberg, C., Shore, D., Giles, S.S., Marr, K.A., Usuka, J., Burch, L.H.,
31
32 Perera, L., Perfect, J.R., Peltz, G., and Schwartz, D.A. (2008). Plasminogen alleles influence
33
34 susceptibility to invasive aspergillosis. *PLoS Genet* 4, e1000101.

35
36
37 Zerbe, C.S., and Holland, S.M. (2005). Disseminated histoplasmosis in persons with interferon-
38
39 gamma receptor 1 deficiency. *Clin Infect Dis* 41, e38-41.

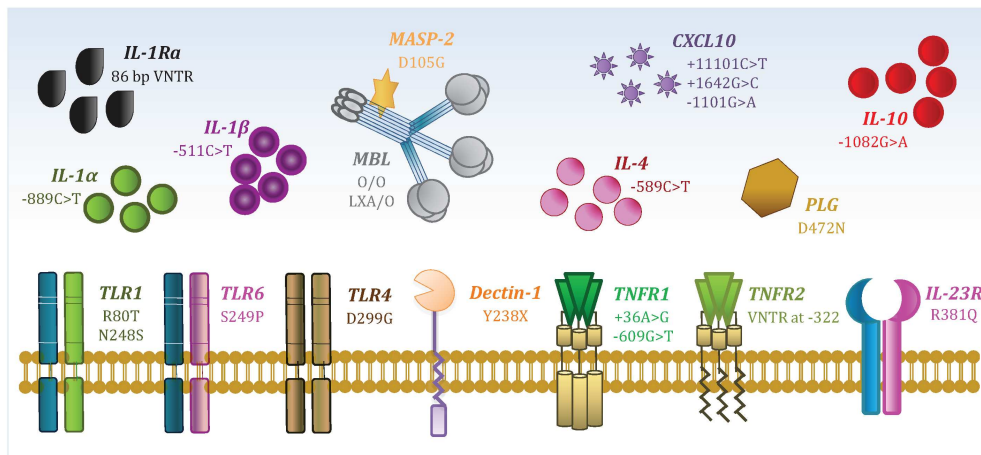


Figure 1. Genetic polymorphisms of pattern recognition receptors (PRRs) and inflammatory mediators associated with susceptibility to invasive fungal diseases.
243x114mm (300 x 300 DPI)