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Review Article

Immunogenetics of invasive aspergillosis

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Invasive aspergillosis is one of the most important infections in hematopoietic stem cell transplant recipients, with an incidence rate of 5–15% and an associated mortality of 30–60%. It remains unclear why certain patients develop invasive aspergillosis while others, undergoing identical transplant regimen and similar post transplant immunosuppression, do not. Over the last decade, pattern recognition receptors such as Toll-like receptors (TLRs) and the C-type lectin receptors (CLRs) have emerged as critical components of the innate immune system. By detecting specific molecular patterns from invading microbes and initiating inflammatory and subsequent adaptive immune responses, pattern recognition receptors are strategically located at the molecular interface of hosts and pathogens. Polymorphisms in pattern recognition receptors and downstream signaling molecules have been associated with increased or decreased susceptibility to infections, suggesting that their detection may have an increasing impact on the treatment and prevention of infectious diseases in the coming years. Infectious risk stratification may be particularly relevant for patients with hematologic malignancies, because of the high prevalence and severity of infections in this population. This review summarizes the innate immune mechanisms involved in *Aspergillus fumigatus* detection and the role of host genetic polymorphisms in susceptibility to invasive aspergillosis.

Keywords *Aspergillus*, innate immunity, genetic polymorphisms, toll-like receptors, C-type lectins, allogeneic stem cell transplantation

Introduction

Invasive aspergillosis (IA) is an important cause of morbidity and mortality in patients with hematological malignancies and prolonged neutropenia. Its incidence depends on multiple factors (such as the type of underlying disease, oncological treatment, antimicrobial prophylaxis) and ranges from 5–15% in patients undergoing intensive myeloablative chemotherapy for acute leukemia or allogeneic hematopoietic stem cell transplantation (HSCT) [1–4]. Although the prognosis of IA has improved with the advent of new effective antifungal drugs, its mortality remains high (30–60%) [1,3–6].

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While early diagnosis is crucial, the non-specific clinical presentation of IA together with the limited sensitivity of current diagnostic methods still makes it particularly challenging. Criteria of the European Organization for Research and Treatment of Cancer and Mycoses Study Group (EORTC-MSG) used to define the probability of IA are often met late in the course of the disease [7]. The optimal management of IA among HSCT patients is under debate [8]. Various strategies have been proposed, including systematic antifungal prophylaxis [9,10], targeted preemptive therapy (which is usually based on the use of circulating fungal antigens and radiological findings) [11,12] and empirical therapy [11,13]. The extended use of prophylactic agents has raised concerns about the emergence of resistance, changing epidemiology of molds, toxicity and costs [9,14]. Indeed, recent studies have highlighted the emergence of non-*Aspergillus* molds such as Zygomycetes which have reduced sensitivity to antifungal agents [6]. The sensitivity and

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specificity of circulating fungal antigens and radiological findings, especially during the early course of infection, are suboptimal [15]. Recent developments in the field of innate immunity and immunogenetics have opened new perspectives for the prevention and management of IA in patients with hematological malignancies. This review discusses the potential use of host genetic profile for patient's risk stratification and individualized preventive strategies.

Innate immunity to *Aspergillus fumigatus*

A. fumigatus is a ubiquitous pathogen that can release large amounts of spores in the air. Due to their small size, conidia formed by asexual reproduction can be inhaled deep into the alveolar spaces [16]. In the immunocompetent host, the innate immune system is usually efficient at clearing conidia before their germination into hyphae, the potentially angioinvasive form of the pathogen. The respiratory epithelium represents a first mechanical barrier (ciliated and mucous-secreting cells) and produces oxidative derivates or other molecules (lactoferrin, chitinase, secretory leukoprotease inhibitor…) which have a direct toxicity on the fungus [17]. In the alveolar compartment, spores interact with macrophages, that contribute to the elimination of the pathogen by phagocytosis and by the production of pro-inflammatory mediators, leading to the subsequent recruitment of polymorphonuclear neutrophils from the lung capillary network, which are essential for fungal clearance [17].

At the molecular level, the innate immune process is triggered by a series of sensors, named 'pattern recognition receptors' (PRRs) located on immune cells that detect microbe-associated molecular patterns (MAMPs) from invading pathogens [18]. PRRs can be classified into four main families, depending on their subcellular localization and biological characteristics, the Toll-like receptors (TLRs), the C-type lectin receptors (CLRs), the RIG-I like receptors (RLRs) and the NOD-like receptors (NLRs, Fig. 1). The PRRs ensure the interface between

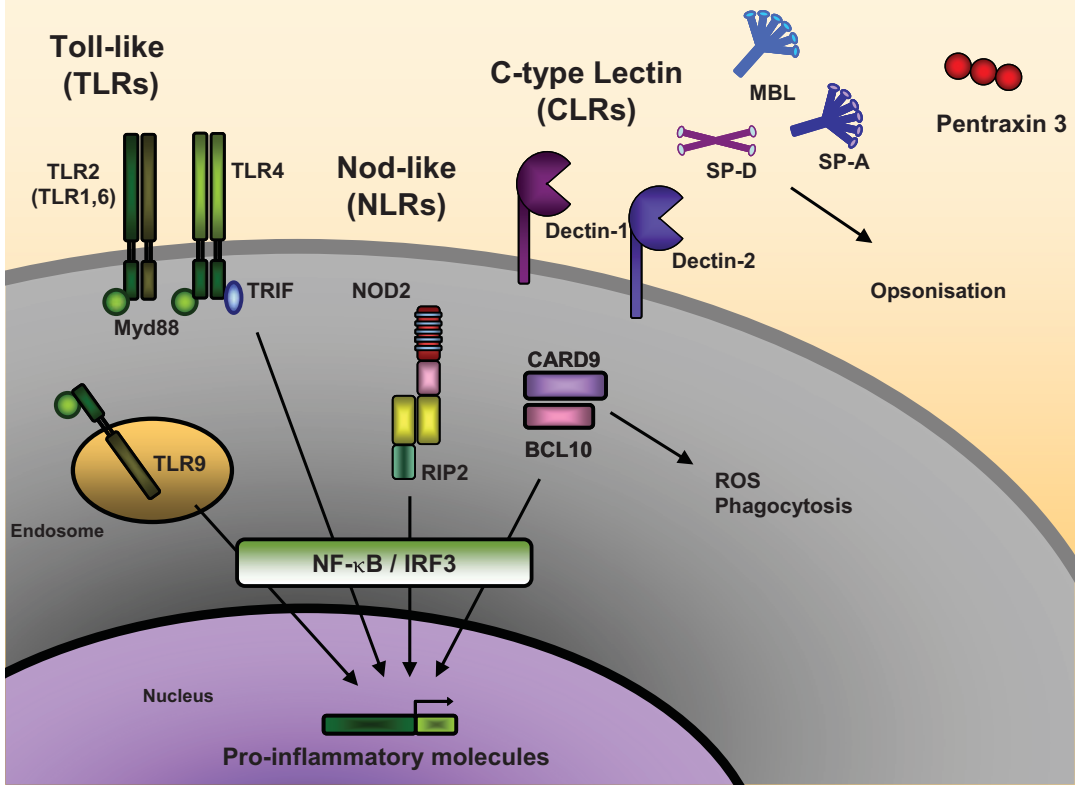

Fig. 1 Pattern recognition receptors involved or potentially involved in the innate immune detection of *Aspergillus fumigatus*. Microbial-associated molecular patterns (MAMPs) from the fungal cell wall are recognized by transmembrane or soluble pattern recognition receptors (PRRs), resulting in the activation of signal-transducing pathways and the production of cytokines and co-stimulatory molecules. MBL, mannose binding lectin; SP-A, surfactant protein A; SP-D, surfactant protein D; MyD88, myeloid differentiation primary response protein; TRIF, TIR-domain-containing adapter-inducing interferon-β; NOD2, nucleotide-binding oligomerization domain containing 2; RIP2, receptor interacting protein 2; CARD9, Caspase recruitment domain-containing protein 9; BCL10, B-cell lymphoma/leukemia 10; ROS, reactive oxygen species; NF-κB, nuclear factor kappa B; IRF3, Interferon regulatory factor 3.

hosts and pathogens and recruit specific intracellular adaptor proteins to trigger signaling pathways, resulting in the activation of transcription factors (such as NF-κB) and the production of cytokines and chemokines that are essential for the innate and subsequent adaptive immune responses. The number of known PRRs and their activators is expanding. Some PRRs such as the collectins (a subgroup of CLRs including mannose-binding lectin and surfactant proteins), complement factors or pentraxin 3 are secreted molecules that act as opsonins [17]. At least two families of PRRs (TLRs and CLRs) have been involved in the innate immune recognition of *Aspergillus fumigatus* (Table 1).

Toll-like receptors (TLRs)

The TLR family in mammals comprises 12 distinct transmembrane proteins, located on the cell surface (TLR1, 2, 4, 5, 6) or within endocytic vesicles (TLR3, 7, 8, 9). The extracellular domain of TLRs is composed of leucin-rich repeat (LRRs) structures that are able to recognize a variety of MAMPs [18–20]. The interaction of TLRs with their specific MAMPs results in the activation of several adaptor proteins, such as the myeloid differentiation primary response protein 88 (MyD88) or the TIR-domain-containing adapter-inducing interferon-β (TRIF), that in turn initiate the activation of transcription factors, such as the

Table 1 Role of Pattern Recognition Receptors (PRRs) and main signaling pathway in the innate immunity against *Aspergillus fumigatus* infection (results of *in vivo/ex vivo/in vitro* studies).

	In vitro/ex vivo experiments				*In vivo* experiments	
PRRs	Macrophages (KO mice) Involved Yes/No	Transfection Involved Yes/No	Inhibition[1] Involved Yes/No	Addition of soluble PRR Involved Yes/No	Lethal model (KO mice) Effect	Sublethal model (KO mice) Effect
Toll-like receptors (TLRs)						
TLR2	Yes [21–26] No [28]	Yes [24–26]	Yes [26,27]	–	–	Absence is deleterious[2] [21,22] No effect[2] [28]
TLR4	Yes [22,23,25,26][3] No [24,26,28][3]	Yes [25] No [24]	Yes [26,27]	–	–	Absence is deleterious[2] [22] No effect[2] [28]
TLR9	Yes [22,31]	Yes [31]	–	–	Absence is protective [22,32]	–
Other TLRs (1,3,5,6,7,8)	–	No [25]	–	–	–	–
MyD88	Yes [22,24] No [28,29]	Yes [85]	–	–	–	Absence is deleterious [22,30] No effect [28]
C-type lectin receptors (CLRs)						
Dectin-1	Yes [47,48]	Yes [47]	Yes [45–47]	–	–	Absence is deleterious [47,48]
DC-SIGN	–	Yes [60]	Yes [61]	–	–	–
Mannose receptor (MR)	–	–	Yes [62][4]	–	–	–
Mannose binding lectins (MBL)	–	–	Yes [111]	–	Presence is protective [52,53]	–
Surfactant protein A (SP-A)	–	–	–	Yes [52,54]	No effect [52,56]	–
Surfactant protein D (SP-D)	–	–	–	Yes [52,54]	Presence is protective [52,56]	–
NOD-like receptors (NLRs)						
NLRP3	-	-	Yes [66]	–	–	–
Others						
Pentraxin-3	Yes [69]	–	Yes [69]	–	Presence is protective [70]	Absence is deleterious [69]
Complement (C5 component)	–	–	–	–	Presence is protective [74]	–

[1]This column regroups different possible modes of inhibition including silencing, antibodies, and purification. [2]Effect on survival has been observed only in TLR2- and TLR4-deficient immunocompromised mice (chemotherapy-induced neutropenia) [21,22], not in immunocompetent mice [22,28]. [3]Stimulation by *Aspergillus* conidia only, not hyphae [22,23,26]. [4]Murine dendritic cells.

nuclear factor kappa B (NFκB) and the activating protein 1 (AP1) (Fig. 1).

All TLRs are expressed by respiratory epithelial cells and alveolar macrophages, and all TLRs but TLR3 are expressed by neutrophils [17]. Two TLRs (TLR2 and TLR4) and the MyD88 signaling pathway are involved in the innate immune detection of *A. fumigatus*, as shown in *in vitro/ex vivo* experiments, including cytokine expression in bone marrow macrophages form wild-type and TLR-deficient mice [21–26], complementation of human cell lines with TLR plasmids [24–26] and gene silencing [26,27] (Table 1). Some discrepant results regarding the role of TLR2 [28], TLR4 [24,26,28] and MyD88 [29] may be explained, at least in part, by changes in the MAMPs exposed to PRRs during the developmental stages of *A. fumigatus* (resting, swollen, germinating conidia and hyphae). Similarly, neutropenic mice deficient in TLR2 and TLR4, as well as the mediator MyD88, were shown to have increased susceptibility to IA in sublethal models of aspergillosis [21,22,30] (Table 1). However, no difference in survival has been observed among immunocompetent mice [22,28]. Some discrepancies may also result from the redundancy of the different PRRs involved in the pathogen recognition. TLR9, another MyD88 dependent-receptor, has also been involved in early detection of *A. fumigatus* probably through its ability to bind the hypomethylated DNA present in the fungus [31]. While a mouse model of IA suggests a protective, albeit modest, effect [22,32], its role in modulating the cytokine response remains complex and poorly elucidated. To our knowledge, one single study using a cell complementation system investigated the role of other TLRs (TLR1, 3, 5, 6, 7, 8), which all seemed not to be involved in the innate immune detection of *A. fumigatus* [25].

The MAMPs from *A. fumigatus* that activate TLR2 and TLR4 are unknown. However, chitin, a major polysaccharide component of the fungal cell wall (present in all fungi including *Aspergillus* spp.), has been shown to induce *in vitro* cytokine production of murine macrophages and acute inflammation in a mouse model through the activation of TLR2 [33]. Other MAMPs of the fungal cell wall have been identified as TLRs activators in fungi other than *Aspergillus* spp. (reviewed in [34]), including phospholipomannans (TLR2) from *Candida albicans* [35], mannans (TLR4) from *C. albicans* and *Saccharomyces cerevisiae* [36], glucuronoxylomannan (TLR4) from *Cryptococcus neoformans* [37], beta-glucan (MyD88) from *Pneumocystis carinii* and *S. cerevisiae* [38,39] and zymosan (TLR2, TLR6) from *S. cerevisiae* [40].

C-type lectin receptors (CLRs)

The large superfamily of CLRs is divided in 17 groups, characterized by the presence of at least one structurally related C-type lectin-like domain. Some CLRs, such as the transmembrane receptors Dectin-1 (group V) and Dectin-2 (group II), the mannose receptor (MR, group VI), DC-SIGN (group II) and collectins (mannose-binding lectin [MBL] and lung surfactant proteins [SP], both group III) are involved in antifungal immunity (reviewed in [41]).

In the CLR family, the best characterized PRR is Dectin-1, which is present on the surface of myeloid cells and is able to bind to the β-glucan component of the fungal cell wall leading to the production of various cytokines and chemokines and the stimulation of phagocytosis [42,43]. Dectin-1 signaling pathway is mediated through the spleen tyrosine kinase (Syk) and the caspase recruitment domain protein 9 (CARD9) [44]. The adjunctive role of some TLRs (TLR2 and 6) may however be required for certain functions [42]. Dectin-1 has been found to be highly expressed in alveolar macrophages and its role in the recognition of *A. fumigatus* through the MAMP β-glucan cell wall component and subsequent activation of the immune system has been shown by *in vitro* and *in vivo* analyses (Table 1) [45–48]. This mechanism of recognition is also dependent from the morphological changes of *A. fumigatus*, being able to distinguish between dormant and potentially threatening spores, as the exposure of β-glucan on the cell wall occurs mainly during the swelling of conidia, which is the first step of germination and transformation to invading hyphae [45–47]. Interestingly, Dectin-1 seems to be required for an effective response to *A. fumigatus* in immunosuppressed as well as in immunocompetent mice [48], which is not the case for TLRs. Prophylactic administration of a fusion protein comprising the extracellular domain of Dectin-1 has been tested in a neutropenic mouse model of IA with promising results [49]. Another transmembrane receptor of the CLRs family, Dectin-2, has been shown to trigger Th17 response to fungal infection [50] and to elicit bone marrow-derived dentritic cells (BMDCs) response to *A. fumigatus* [51].

Collectins are soluble CLRs characterized by the presence of collagen. Their C-terminal region consists of a carbohydrate-recognition domain (CRD) which has the ability to interact with a wide range of microbial molecules and to activate the innate immune system [41]. Collectins such as the lung surfactant proteins (SP-A and SP-D) and the mannose-binding lectin (MBL) were shown to bind *in vitro* to *A. fumigatus* conidia in the presence of calcium acting as osponins for their phagocytosis by alveolar macrophages and neutrophils [52]. The works of Madan *et al.* have emphasized their role in the innate immune response against *A. fumigatus* antigens or allergens and their therapeutic potential in murine models of invasive aspergillosis and allergic bronchopulmonary aspergillosis (Table 1) [52–59].

DC-SIGN is expressed exclusively on the surface of dendritic cells and a subset of macrophages through an IL-4 dependent manner and is able to recognize a wide spectrum of microbial agents [41]. DC-SIGN recognizes carbohydrates such as high-mannose structures. Some works have highlighted its ability to bind to *A. fumigatus* conidia and mediate their internalization by dendritic cells and macrophages triggering their maturation (Table 1) [60,61]. It has been suggested that *A. fumigatus* may use the DC-SIGN functions as a mean to escape immune recognition: increased DC-SIGN expression mediated by IL-4 may promote alternative activation of macrophages with reduced antifungal activity facilitating the development of IA by persistence and germination of intracellular conidia [61].

The mannose receptor (MR) is a transmembrane endocytic receptor mainly expressed in macrophages and dendritic cells. Because of its ability to bind terminal mannose, fucose or N-acetyl glucosamine, MR is implicated in the recognition and immune response to various fungi such as *C. albicans*, *P. carinii* and *C. neoformans* [17,41]. One study suggests its role in the recognition and internalization of *A. fumigatus* conidia by murine dendritic cells (Table 1) [62]. This function has not been confirmed in human dendritic cells. A study using human Langerhans cells suggests their ability to recognize *A. fumigatus* through a CLR with galactomannan specificity distinct from mannose receptor [63].

NOD-like receptors (NLRs) and RIG-I like receptors (RLRs)

NLRs (Nucleotide-binding domain, Leucin-Rich repeat containing) are conserved cytoplasmic proteins encoded by 22 genes in humans that are involved in microbial recognition and responses to stress. Their action is mediated through the activation of multiprotein complexes (including caspases) called inflammasomes that trigger the maturation of cytokines [64]. They can be subdivided in three subfamilies: NLRC (previously called NOD), NLRP and IPAF [64].

Among NLRCs, NOD-2 is a receptor expressed essentially by leukocytes, dendritic cells and epithelial cells which is well-known for its ability to bind to the peptidogylcan of gram-negative and gram-positive bacteria. Its role in the innate immune response to *A. fumigatus* has been suggested by a recent study documenting *in vitro* and *in vivo* increased NOD-2 levels after stimulation by conidia, although direct evidence is lacking [65]. A study in human monocyte demonstrated that the NLRP3 inflammasome is activated by *A. fumigatus* hyphae leading to IL-1β release [66]. NLRP3 signaling seems to rely on the Syk tyrosine kinase pathway, also activated by Dectin-1 and distinct from the MyD88 pathway of TLRs [66]. RLRs are cytoplasmic RNA helicases, including RIG-I, MDA5 and LGP2, which are mainly involved in the recognition of viral RNA [67]. Their potential role in the innate immune response to fungal pathogens has not been established.

Other PRRs

In addition to these important families of PRRs, other soluble molecules have been recognized as important mediators at the interface between host and pathogen. Some of them may be involved in the immune response to *A. fumigatus* acting as opsonins and facilitating their elimination by the host's immune system, such as pentraxins or proteins of the complement and the fibrinolytic pathways.

Pentraxins are conserved polymeric proteins representing a superfamily divided into two groups: short and long pentraxins. Pentraxin 3 (PTX3) is a long pentraxin produced by sentinel cells and released in response to cytokines in inflammatory conditions that can bind to various microbial agents including *A. fumigatus* [68,69]. Lack of PTX3 is associated with defective recognition of *A. fumigatus* conidia by alveolar macrophages and dendritic cells and with decreased survival in a murine model of invasive pulmonary aspergillosis (Table 1) [69]. The potential role of this soluble receptor in the treatment of invasive aspergillosis has also been studied with promising results suggesting a potentiating effect when combined with amphotericin B [70].

Proteins of the complement have many antimicrobial properties including inflammation, opsonization and pathogen destruction. The observation in 1989 that spores of *A. fumigatus* were more susceptible to killing by alveolar macrophages when serum has been heated to 56°C suggested a role of heat-labile serum components of the complement in antifungal defences [71]. Interactions of *A. fumigatus* with the complement component C3 leading to the cleavage of C3 and the activation of an alternative pathway that facilitates phagocytosis, as well as the ability of the fungus to escape the activation of the complement, have been described [17,72,73]. An important role of the component C5 in the resistance to IA has also been suggested by a mouse model [74].

As opposed to most PRRs, which were mainly discovered by classical biological approaches, the identification of plasminogen as an important contributor to the immune defences against *A. fumigatus* resulted from a large genetic mapping study [75]. The role of plasminogen in the inflammatory response and mechanisms of microbial pathogenicity such as invasion or dissemination has been widely described [76]. The demonstration that this protein may bind *in vitro* to *A. fumigatus* suggests its role in some mechanisms of pathogenicity of the fungus, such as hemorrhage and tissue damage associated with IA [75].

Cytokines and chemokines. These molecules secreted by sentinel cells are not PRRs, but the immunoregluatory effectors of the immune system and the ultimate product resulting from the interactions of MAMPs and PRRs.

Tumor Necrosis Factor alpha (TNFα) stimulates various functions of polymorphonuclear leukocytes including secretion of other cytokines, phagocytosis, oxidative respiratory burst and degranulation. Its key role in the inflammatory response against *A. fumigatus* has been demonstrated by *in vitro* and *in vivo* studies [77–79]. Various interleukines are secreted in response to *A. fumigatus*. While some of them have a protective pro-inflammatory role (IL-1, IL-6, IL-12 or IL-15), others seem to impede the host defences or to have controversial effects [17,80]. For instance, IL-10 suppresses the antifungal activity of macrophages against *A. fumigatus*, while increasing phagocytosis [17,81]. A detrimental effect of the IL-23/IL-17 pathway in the inflammatory response and resistance against *A. fumigatus* has recently been highlighted by *in vitro* analyses and a murine model of invasive aspergillosis [82]. Chemokines, such as KC and MIP-2, have been shown to promote neutrophil recruitment in a murine model of IA [79,83,84]. MIP-2 is considered to be the murine homolog of IL-8 (not present in mice), a potent inflammatory mediator which is secreted by human respiratory cells and neutrophils in response to *A. fumigatus* [23,85]. Chemokines receptors, such as CCR1, CCR6 and CXCR2, have also been shown to play a role in host defences against *A. fumigatus* [86–88].

Genetic polymorphisms of pattern recognition receptors and risk of aspergillosis

Invasive aspergillosis represents a particular interest for immunogenetic studies as it affects patients from a specific, relatively homogenous population (i.e., allogeneic hematopoietic stem cell transplant recipients) that can benefit from preventive strategies. A relatively large number of studies have investigated the association of numerous SNPs or other genetic variations of different pattern recognition receptors (Table 2) or inflammatory mediators (cytokines, chemokines, Table 3) and an increased risk of aspergillosis.

Toll-like receptors (TLR)

A study identified a donor haplotype in TLR4 (containing the D299G and T399I SNPs) present at a frequency of 6% in the Caucasian population as a predictor of IA (22% vs 5% at 6 months, $P = 0.002$) [89]. This association was confirmed in a large (>300 patients) validation study. The presence of this TLR4 haplotype and/or of a CMV positive serostatus (donor or recipient) compared to negative results for both CMV and the TLR4 haplotype increased the 3-year cumulative incidence of IA (12% vs 1%, $P = 0.02$) and death not related to relapse (35% vs 22%, $P = 0.02$). Although TLR4 has been shown to detect *A. fumigatus* [22,23,25–27], the functional role of the D299G and T399I SNPs remains partially controversial [90]. The interaction of TLR4 with other pathogens such as CMV [91] or antifungal drugs [92] has been proposed as alternative explanations by which TLR4 SNPs influence susceptibility to IA. However, TLR4 D299G and T399I SNPs have been associated with susceptibility to aspergillosis in non-HSCT patients, who are not susceptible to CMV disease and usually did not receive antifungal agents prior to diagnosis [93]. Furthermore, CMV disease and TLR4 SNPs seem to be independent factors for the risk of IA among HSCT recipients [89].

Another study reported an association of the same SNP with fungal colonization in HSCT recipients, although this was not associated with an increased risk of IA in the small subset of colonized patients [94]. Discrepancies between studies may be explained by several factors, such as different types of patients, differential assessment of demographic or clinical co-factors into multivariate models, and the power to detect associations. An increased risk of IA has also been associated with polymorphisms of TLR1 and TLR6 in HSCT recipients [95]. It is noteworthy that no genetic polymorphism predisposing to aspergillosis has been identified in TLR2 up to now, although it has been investigated in some studies [89,93,94]. However, the absence of genetic association should always be interpreted within the limited power of existing studies.

C-type lectin receptors (CLRs)

Some studies have investigated the role of polymorphisms in MBL and lung surfactant proteins in the development of aspergillosis (Table 2) [75,96–100]. Most of them addressed the issue of chronic pulmonary aspergillosis and not IA, comparing small series of cases with a control group. Although some of them suggest an association of the disease with some SNPs, the very limited sample size (<25 cases of aspergillosis) does not allow drawing firm conclusions. However, a role of the 868 C/T polymorphism in the MBL gene in predisposing to chronic pulmonary aspergillosis is supported by two small studies showing concordant results [96,100]. The presence of SNPs in other CLRs that may affect the host's response to *A. fumigatus* has not been investigated up to now. The recent discovery of the association of an early stop polymorphism in dectin-1 with an increased susceptibility to colonization with *Candida* species deserves further investigations with respect to IA [101].

Table 2 Association between genetic polymorphisms of pattern recognition receptors (PRRs) of the innate immune system and aspergillosis.

First author, year, journal (reference)	Patients, number, ethnicity (if specified)	Type of aspergillosis Total number (proven/ probable)	Genes tested	Genes involved	rs Number	Nucleotide[1]	Amino acid[1]	Association OR (95% CI), *P* value
Kesh, 2005	HSCT (R)	IA	TLR1	TLR1	rs5743611	239 G/C	R80T	1.2 (1.0–1.5), $P = 0.04$
Ann NY Acad Sci [95]	127	22 (22)	TLR4	TLR1 and	rs4833095	743 A/G and	N248S and	1.2 (1.0–1.5), $P = 0.02$
			TLR6	TLR6	rs5743810	745 C/T	S249P	
					rs5743611	239 G/C or	R80T or (N248S	1.3 (1.1–1.5), $P < 0.001$
					rs4833095	(743 A/G and	and S249P)	
					rs5743810	745 C/T)		
Bochud, 2008	HSCT (D/R)	IA	TLR2	TLR4	rs4986790	1063 A/G &	D299G &	6.2 (2.0–19.3), $P = 0.002$ (D)
N Engl J Med [89]	336, Caucasian	33 (33)	TLR3		rs4986790	1363 C/T[2]	T399I[2]	(discovery study)
	(validation group, $N = 366$)	(103 validation gr.)	TLR4					2.5 (1.2–5.4), $P = 0.02$ (D)
			TLR9					(validation study)
Carvalho, 2008	Immunocompetent	CPA	TLR2	TLR4	rs4986790	1063 A/G	D299G	3.5 (1.5–8.1), $P = 0.003$
J Infect Dis [93]	40 (80 controls)	40	TLR4					
			TLR9					
	Immunocompetent	ABPA	TLR2	TLR9	rs5743836	–1237 C/T	Promotor	2.5 (1.0–6.1), $P = 0.04$
	22 (80 controls)	22	TLR4					
			TLR9					
Carvalho, 2009	HSCT (R)	IA	TLR2	TLR4	rs4986790	1063 A/G &	D299G &	0.2 (0.1–0.9), $P = 0.03$
Exp Hematol [94]	58	34 (34)	TLR4			1363 C/T[2]	T399I[2]	
			TLR9					
Crosdale, 2001	Immunocompetent	CPA	MBL	MBL	rs5030737	868 C/T	C52R	4.9 (1.3–18.0), $P = 0.02$
J Infect Dis [96]	10 (82 controls), Caucasian	10						
Granell, 2006	HSCT (D/R)	IA	MBL	MBL	NA	MBL-« low	NA	7.3 (1.9–27.3), $P = 0.003$ (D)
Exp Hematol [97]	121, Caucasian	16 (8)	MASP-2			genotype »[3]		
				MASP-2	rs72550870	380 A/C	D120G[4]	6.4 (2.0–20.6), $P = 0.002$ (R)
Kaur, 2006	Immunocompetent	ABPA	MBL	MBL	rs36203921	1011 A/G	Intron	8.2 (2.8–23.6), $P < 0.003$
Clin Exp Immunol [98]	11 (84 controls), Indian	11						
Vaid, 2007	Immunocompetent	CPA	MBL	MBL	rs5030737	868 C/T	C52R	3.3 (1.2–8.9), $P = 0.02$
Clin Chem Lab Med [100]	15 (82 controls), Caucasian	15	SP-A1					
			SP-A2					
Saxena, 2003	Immunocompetent	ABPA	SP-A1	SP-A2	rs17886221	1660 A/G	R94R	4.8 (1.2–18.5), $P = 0.02$
J Allergy Clin Immunol [99]	22 (23 controls), Indian	22	SP-A2		rs17886395	and 1649 C/G	and A91P	10.4 (1.6–66.9), $P = 0.008$[5]
Zaas, 2008	HSCT (R)	IA	PLG	PLG	rs4252125	28904 G/A	D472N	3.0 (1.5–6.1) (DN vs DD)
PLoS Genet [75]	194	59 (59)						5.6 (1.9–16.5) (NN vs DD)
								$P < 0.001$ (both)

HSCT, allogeneic hematopoietic stem cell transplant recipients; R, recipient; D, donor; IA, invasive aspergillosis; CPA, chronic pulmonary aspergillosis; ABPA, allergic bronchopulmonary aspergillosis; NA, data not available; MBL, mannose binding lectin; MASP, mannose binding lectin associated serine protease; SP-A, surfactant protein A; PLG, plasminogen.

Only significant associations ($P \leq 0.05$) have been mentioned. [1]Major allele is shown first. Effect allele is underlined. [2]Both SNPs are in strong linkage disequilibrium ($R^2 = 0.96$). [3]« Low genotypes » correspond to a group of haplotypes denoted by letters (O/O or LXA/O) as described in reference [112]. [4]Based on NCBI, some authors also reported D105G depending on aminoacid count with or without including the signal sequence [106]. [5]Stronger association if both alleles present.

Table 3 Association between genetic polymorphisms of cytokines (or cytokines receptors) and aspergillosis.

First author, year, journal (reference)	Patients, number, ethnicity (if specified)	Type of aspergillosis Total number (proven/probable)	Genes tested	Genes involved	rs Number	Nucleotide[1]	Amino acid[1]	Association OR (95% CI), *P* value
Seo, 2005	HSCT (R)	IA	IL-10	IL-10	rs1800896	–1082 A/G and	Promotor	9.3 (1.6–52.8), $P = 0.01$
Bone Marrow Transplant [109]	105, Asian	9 (5)			rs1800871	–819 C/T and		
					rs1800872	–592 A/C		
Sainz, 2007	Hematological	IA	IL-10	IL-10	rs1800896	–1082 A/G	Promotor	4.5 (1.6–12.9), $P = 0.001$
Immunol Lett [105]	120 (124 controls) Caucasian	59 (NA)						
Brouard, 2008	Cystic fibrosis	ABPA	IL-10	IL-10	rs1800896	–1082 A/G	Promotor	1.7 (0.6–4.4), $P = 0.01$
J Infect Dis [102]	378	27						
Sambatakou, 2006	Immunocompetent	CPA, ABPA	IL-10	IL-15	NA	13689 T/A	Exon 6	2.4 (1.3–4.2), $P = 0.003$
Int J Immunogenet [108]	39 (65–330 controls)	24	IL-15	TNF-α	rs1800629	–308 G/A	Promoter	$P < 0.01$
			TNF-α					
			TGF-β1					
			IFN-γ					
Sainz, 2007	Hematological	IA	TNF-α	TNFR2	VNTR	VNTR (−322)	Promotor	2.5 (1.1–5.0), $P = 0.03$
Hum Immunol [106]	102 (124 controls) Caucasian	54 (NA)	LT-α					
			TNFR2					
Sainz, 2008	Hematological	IA	IL-1α	IL-1α	rs1800587	–889 C/T and	Promotor	15.4 (1.4–171.2), $P = 0.02$
J Clin Immunol [107]	110 (148 controls)	59 (NA)	IL-1β	IL-1β	rs1143627	–511 C/T and		
			IL-1Ra	IL-1Ra	VNTR	VNTR2		
Mezger, 2008	HSCT (R)	IA	CXCL 10	CXCL 10	rs1554013	11101 C/T	Intron	2.2 (1.2–3.8), $P = 0.007$
Blood [104]	139, Caucasian	81 (81)			rs3921	1642 C/G	3'UTR	2.6 (1.4–5.0), $P = 0.003$
					rs4257674	–1101 A/G	Promotor	2.8 (1.6–5.2), $P = 0.001$
Knutsen, 2006	Cystic fibrosis/asthma	ABPA	IL-4Rα	IL-4Rα	rs1805010	4679 A /G	I75V	$P = 0.008$ (heterozygous)
Clin Mol Allergy [103]	40 (56 controls)	40						$P < 0.001$ (homozygous)

HSCT, allogeneic hematopoietic stem cell transplant recipients; R, recipient; IA, invasive aspergillosis; CPA, chronic pulmonary aspergillosis; ABPA, allergic bronchopulmonary aspergillosis; NA, data not available; IL, interleukine; TNF, tumor necrosis factor; LT, lymphotoxin; CXCL, chemokine (C-X-C motif) ligand; TNFR, tumor necrosis factor receptor; VNTR, variable nucleotide tandem repeats. Only significant associations ($P \leq 0.05$) have been mentioned. [1]Major allele is shown first. Effect allele is underlined.

Other PRRs

Using a genetic mapping model approach in a murine model, Zaas *et al*. have identified a SNP in the gene encoding plasminogen that affected IA outcome in mice [75]. The association between this SNP and an increased susceptibility to IA was confirmed in a large cohort study of HSCT recipients [75]. Such an interesting approach may allow the identification of new PRRs and related polymorphisms improving our understanding of the pathogenesis of *A. fumigatus*.

Cytokines and chemokines

Small association studies suggest that different SNPs in the genes encoding various cytokines and chemokines or their receptors may predispose to aspergillosis (Table 3) [102–109]. Concordant results suggesting an increased risk of aspergillosis related to the presence of a SNP in the encoding gene of interleukine 10 (G/A at position -1082) were described by two separate groups of investigators [102,105]. These results deserve further investigations in larger data sets.

Synthesis and future perspectives

Over the last decade, PRRs appeared as major contributors of the immune responses to *A. fumigatus*. Growing evidence supports the role of TLR2, TLR4 and dectin-1 in the detection of the fungus. Furthermore, a number of studies investigated the association of polymorphisms in PRRs or cytokine genes with susceptibility to aspergillosis. Certain polymorphisms are emerging as real risk factors, because the associations have been validated and/or functional consequences of the polymorphism have been clearly demonstrated. These data suggest that genetic markers may be used for infectious risk stratification in the coming years.

However, many genetic association studies still need further validation, as their quality and methodological approaches are quite variable. Most common limitations include limited sample size in retrospective studies, use of inappropriate or ill-defined controls (no reporting or adjustment for underlying diseases, type of transplant, degree and duration of immunosuppression, antifungal drugs or concomitant infections), absence of time-dependent analyses, no reporting on ethnicity, lack of correction for multiple testing with respect to the multiplicity of markers, absence of replication studies to validate new associations and over-interpretation of negative results in underpowered studies. Furthermore, many studies have focused on one or a limited number of genetic markers, without accounting for the others. Due to the ethnic diversity, polymorphisms identified as risk factors in a given population may not apply to another one [110].

Larger studies with detailed clinical data will help to precisely determine the individual contribution of several individual polymorphisms to the risk of developing the disease. New approaches combining the use of well defined host genetic markers, more sensitive and specific serologic tests and improved radiological surveillance may significantly improve the management of IA in the future.

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