12 Toll-Like Receptors and Fungal Recognition

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I. Introduction

More than 100 000 fungal species exist and humans are commonly exposed to them. Despite this permanent encounter, only very few fungi are pathogenic to humans. Superficial skin infections caused by dermatophytes are common, but usually not severe and therefore not within the scope of this review. In contrast, some fungi are able to establish rare, but severe and life-threatening systemic mycoses. These fungal pathogens belong either to the so-called 'dimorphic fungi' (e.g. *Histoplasma capsulatum*, *Paracoccidioides brasiliensis*, *Blastomyces dermatitidis*, *Coccidioides immitis*) or are opportunistic fungal pathogens, like *Candida albicans*, *Aspergillus fumigatus* and *Cryptococcus neoformans*.

Infections with dimorphic fungi commonly start with the inhalation of fungal spores. The infectious morphotype is the yeast form, while hyphal growth is found under in vitro conditions at temperatures below 37 °C. Dimorphic fungi are endemic in the Americas and can cause systemic infections even in immunocompetent individuals. Opportunistic fungal pathogens can also cause systemic mycoses, but only in individuals with severe immunological deficiencies, like HIV patients, transplant recipients or patients with certain forms of cancer. The mortality rates associated with systemic mycoses are generally high, due to the still suboptimal diagnostic and/or therapeutic options. In addition, the number of patients at risk to acquire infections by opportunistic fungi is constantly rising as a result of medical progress, and therefore opportunistic fungal infections represent a severe problem in modern medicine.

II. The Innate Immune Response

Microbial infections are a common, serious threat to multicellular organisms and forced them to develop means to counteract this challenge. The sophisticated immune system found in vertebrates comprises two major branches: the adaptive and the innate immune system. In an adaptive immune response exposure to microorganisms leads to selection and clonal expansion of highly specific B- and T-cells, which help to eliminate the respective microbial pathogen and to establish a highly specific immunological memory. Although this process is extremely efficient, it requires several days in which the host depends on the alternative and evolutionary more ancient defense strategies of the innate immune system. This immune response relies on a limited number of germ line encoded, cell-bound or soluble receptors that detect microbes and induce their engulfment and elimination by a set of specialized phagocytic cells. These phagocytes, e.g. macrophages, neutrophils and dendritic cells, are not only responsible for the fast clearance of invading microbes, but are also required for the presentation of antigens, a feature which drives the process of clonal selection and expansion of B- and T-cells. This demonstrates the instructive role of the innate immune system

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for the adaptive immunity and underlines the interdependence of both branches of the immune system.

Those parts of the host which are exposed to the outside environment and are therefore especially susceptible to microbial infections, e.g. the gut or the lung, require an enhanced surveillance by the immune system. The specificity of the innate immune response has to be broad to deal with a wide variety of microbial challenges, but it relies only on a very limited number of receptors. Our knowledge on the molecular basis of innate immune recognition has increased substantially over the past decade. A very important step in this process was made by Medzhitov and Janeway (2000), who were the first to describe the basic principle of the innate immune recognition. According to this concept, germ line encoded 'pattern recognition receptors' (PRRs) recognize microbial patterns, which are commonly indicative for a certain subgroup of microorganisms. These 'pathogen-associated molecular patterns' (PAMPs) are structural components that are essential for the microbial survival and therefore conserved among many microorganisms (Table 12.1). An archetype of such a PAMP is lipopolysaccharide (LPS), which is an essential component of the outer membrane of Gram-negative bacteria and recognized by Toll-like receptor (TLR) 4 (Poltorak et al. 1998). As for LPS, such PAMPs are usually not pathogen-specific, but conserved between pathogenic and non-pathogenic microorganisms. It was therefore recently suggested to rename them 'microbe-associated molecular patterns' (MAMPs; Didierlaurent et al. 2002), but PAMP is still the commonly used term. Recognition of PAMPs by soluble or cell-bound PRRs may lead to different kinds of responses, depending for instance on the cell type involved. In general, enhanced phagocytosis and induction of an inflammatory response represent the major mechanisms that are triggered and that mediate the clearance of infection. However, activation of PRRs may also lead to the production of anti-inflammatory cytokines, like IL-10, which can result in an attenuation of an immune response. During infection signals derived from invading microorganisms are processed by several types of immune and non-immune cells, e.g. epithelial or endothelial cells, and this leads to a complex and orchestrated immune response that in its entirety is not easy to analyze.

The struggle of multicellular organisms with their microbial challengers started very early in evolution, and the horseshoe crab *Limulus polyphemus*, a so-called 'living fossil', represents a paradigm for an ancient immune system that already comprises elements and mechanisms characteristically found in the mammalian innate immune response (Iwanga and Lee 2005). Limulus recognizes LPS and β(1–3) glucan by soluble lectins that can activate an immune response cascade. The two PRRs, designated Factor C and Factor G, enable specific detection of Gram-negative bacteria and fungi. The horseshoe crab responds to the infection with a specific hemolyph clotting reaction. This highly specific and sensitive response is nowadays used in commercially available tests for detection and quantification of LPS and β(1–3) glucan (Muta 2006).

Table 12.1. Ligands recognized by human Toll-like receptors (TLRs). *LBP* lipopolysaccharide (LPS)-binding protein, *MD-2* myeloid differentiation protein 2, *PAMPs* pathogen-associated molecular patterns, * endogenous ligands

Toll-like receptor	Co-factors	Localization	PAMPs
TLR1	TLR2	Surface	Triacylated lipopeptides
TLR2		Surface	Peptidogycan, lipoproteins, lipopeptides, atypical LPS
TLR2	Dectin-1	Surface	Zymosan
TLR3		Endosome (?)	Double-stranded viral RNA
TLR4	MD-2, LBP	Surface	LPS, mannans, glucuronoxylmannan, glycoinositolphospholipids, viral fusion proteins, taxol, heat shock proteins*, fibrinogen*, fibronectin*
TLR5		Surface	Bacterial flagellin
TLR6	TLR2	Surface	Diacylated lipopeptides
TLR7		Endosome	Imidazoquinoline, single-stranded viral RNA
TLR8		Endosome (?)	Imidazoquinoline, single-stranded viral RNA
TLR9		Endosome	Non-methylated CpG DNA, chromatine immune complexes*
TLR10		Surface (?)	Unknown

III. The Family of Toll-Like Receptors

The Toll-like receptors (TLRs) represent the paradigmatic example of a PRR. The impulse for the identification of these receptors in humans came from work on a *Drosophila melanogaster* gene cassette that was originally shown to regulate the dorsoventral patterning of the fruit fly (Anderson et al. 1985). Later the *toll* gene, which is part of this cassette, turned out to be essentially required for protection against fungal infections (Lemaitre et al. 1996). Toll is a transmembrane protein comprising an extracellular domain with several leucin-rich repeats (LRRs) and an intracellular domain that shares significant homology with the intracellular domain of the human IL-1 receptor (Hultmark 1994). This Toll/IL-1 receptor (TIR) domain is required for the intracellular signalling of both receptors. The functional importance of Toll in the *Drosophila* immune response sparked the search for homologous human proteins (Rock et al. 1998). Eleven homologous sequences were identified in the human genome, of which ten encode for a functional protein. These proteins have been designated Toll-like receptors since they share all characteristic structural elements with *Drosophila* Toll.

Leucin-rich repeats are well characterized modules which are often engaged in protein-ligand interactions (Kobe and Deisenhofer 1995) and the LRRs present in the members of the TLR family are therefore likely (and in part proven) candidates for recognition of distinct PAMPs. The concave surface of the horseshoe-like structure of the LRR domains likely provides the interface for the interaction with the PAMP ligands. The recent elucidation of the structure of the ectodomain of human TLR3 furthermore revealed that the regular surface of the LRRs is in this case disrupted by two insertions that seem to be an essential element of the two putative PAMP binding sites of this receptor (Ben et al. 2006).

Ligation of PAMPs to the TLR ectodomains facilitates receptor dimerization, which represents a very early step in the signal transduction mechanism. The further signalling events require the myeloid differentiation marker protein MyD88 which also contains a TIR domain (Hultmark 1994) that interacts with the cytoplasmic TIR domain of the TLRs (Xu et al. 2000). The 'TIR domain-containing adaptor protein' TIRAP6 is involved in the MyD88-dependent signalling pathway of TLR2 and TLR4, whereas the 'TIR domain-containing adaptor inducing interferon-β' (TRIF) and the 'TRIF-related adaptor molecule' (TRAM) are involved in the MyD88-independent signalling of TLR3 and TLR4, leading to specific immunological responses upon each TLR stimulation (Fig. 12.1).

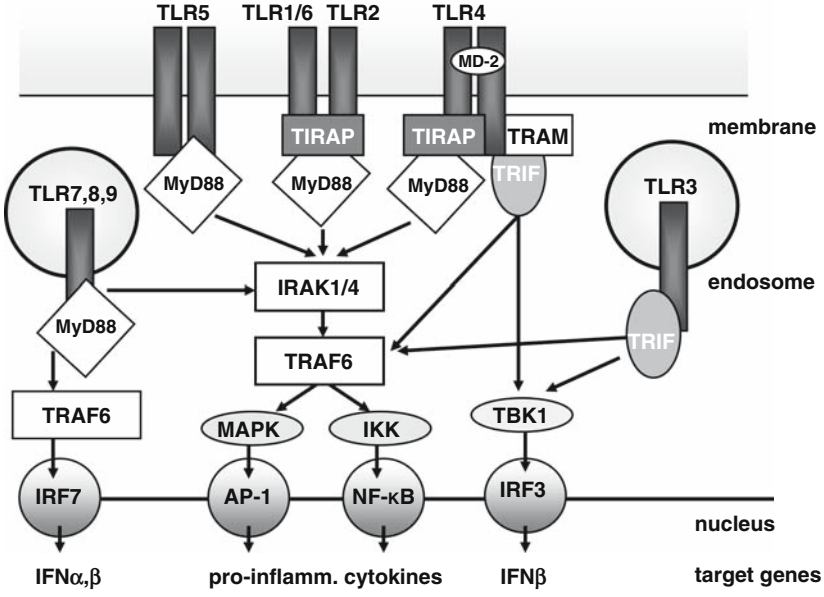

Fig. 12.1. TLR signalling pathways. *IRAK* Interleukin-1 receptor I-associated kinase, *IRF* IFN regulatory factor, *IFN* interferon, *MD-2* myeloid differentiation protein 2, *MyD88* myeloid differentiation marker 88, *TBK1* TANK-binding kinase 1, *TIR* Toll/IL-1 receptor, *TIRAP* TIR domain-containing adaptor protein, *TNF* tumor necrosis factor, *TRAF* TNF receptor-associated factor, *TRAM* TRIF-related adaptor molecule, TRIF TIR domain-containing adaptor inducing IFNβ

The TRIF-independent pathways converge at a complex comprising the 'TNF receptor-associated factor 6' (TRAF6) and the 'interleukin-1 receptor-associated kinase' (IRAK). The signalling downstream of the TRAF6/IRAK complex is highly conserved and culminates in the translocation of the nuclear factors NF-κB and AP-1 to the nucleus and the subsequent activation of certain sets of genes implicated in an inflammatory immune response (Akira and Takeda 2004). A very similar signalling cascade is triggered by the Toll receptor in *Drosophila* (Silverman and Maniatis 2001), with one interesting difference to the situation in mammals: TLRs are supposed to directly interact with their respective PAMPs, whereas Toll binds the *Drosophila* protein Spätzle, which is present in the *Drosphila* body fluid and serves as a kind of extracellular indicator protein. To be able to bind to Toll, Spätzle has to be processed by a serine protease that is activated during infection (Ligoxygakis et al. 2002). Thus, Toll does not function as a PRR according to the concept of Medzhitov and Janeway, but is merely a building block in the signalling cascade that originates from a still unknown *Drosphila* molecule that senses the microbial infection.

Of the ten functional human TLRs at least some seem to be able to recognize several, biochemically diverse PAMPs (Kawai and Akira 2005). This flexible interaction with different ligands might be facilitated by the engagement of different binding sites on one receptor (Ben et al. 2006) and/or the ability of certain TLRs to form functional heterodimers, e.g. TLR1/TLR2 and TLR2/TLR6 (Ozinsky et al. 2000). Since several PRRs are usually engaged in recognition of a certain microbial pathogen, the resulting cross-talk between these receptors and their signalling pathways may also contribute to the broad spectrum of possible outcomes.

To our current knowledge, TLR2 and TLR4 are the most promiscuous TLRs and both seem to be particularly important for the recognition of PAMPs that are localized on microbial surfaces. TLR2- and TLR4-signalling pathways have been shown to interact in a synergistic way to enhance and balance an inflammatory response (Sato et al. 2000). Depending on their specific function members of the TLR family are differentially expressed and localized within different types of cells. TLR3, TLR7 and TLR9, which recognize double-stranded RNA and so-called CpG DNA motifs (Hemmi et al. 2000; Alexopoulou et al. 2001; Lund et al. 2004), are exclusively found in endosomes, while TLR2 and TLR4, which either alone or in combination have been implicated in the immune response to several bacterial and fungal pathogens, are found on the cellular surface (Takeda and Akira 2005). However, depending on the cell type, both receptors may also reside either in part or exclusively in certain intracellular compartments (Hornef et al. 2002; Meng et al. 2004). During infection these TLRs may re-localize to phagosomes containing engulfed microbes and the phagosome is likely to represent the principal stage for the recognition of PAMPs by TLR2 and TLR4 (Underhill and Gantner 2004).

TLR-mediated recognition of microbes by macrophages usually leads to the release of pro-inflammatory molecules, which recruit and activate neutrophils and other macrophages (Takeda and Akira 2005). The pattern of the released cytokines will also drive the immune response towards an either T helper cell (Th) 1- or Th2-like type. Dendritic cells are of particular importance in this process, as they stand at the cross-roads of innate and adaptive immunity and their response decisively determines whether an anti-fungal immune response is protective or not (Romani et al. 2002; Mazzoni and Segal 2004). The TLR-induced modulation of phagocytosis by TLR signalling (Underhill and Gantner 2004; Luther et al. 2007) and the production of anti-microbial products, like reactive oxygen species or nitric oxide, provide further and more direct means for the clearance of microbial infections. In general, an optimal anti-fungal immune response combines oxidative and non-oxidative mechanisms, the latter consisting of an efficient phagosomal maturation, the release of anti-fungal effector molecules by degranulation and the sequestration of iron.

Macrophages and neutrophils are the main effector cells responsible for the elimination of fungal pathogens; and both have been shown to engulf single fungal cells and to produce reactive oxygen species for microbial killing. During fungal infections, neutrophils are of particular importance for the elimination of multicellular fungal organisms that escape phagocytosis, but are susceptible to effector molecules released by degranulation (Schaffner et al. 1982).

As outlined above, an immune response has to be regulated in a way that leads to the activation of those effector cells and mechanisms required for the elimination of the respective pathogen.

However, during infection, the inflammatory response has to be limited and controlled to avoid deleterious side effects and after elimination of the invading microorganism the immune response has to be brought back to homeostasis by anti-inflammatory mechanisms. Interestingly, certain TLRs have also been shown to participate in this process (Netea et al. 2006b) and other means exist which are capable of down-regulating a sustained TLR response, for which LPS tolerance is the most prominent example (Takeda and Akira 2005). In conclusion, TLRs can be engaged in the activation, modulation and repression of an inflammatory response.

IV. The Role of Non-TLR Pattern Recognition Receptors in Antifungal Immune Responses

The complement system comprises a complex, tightly regulated network of a multitude of soluble factors as well as a set of corresponding cell bound receptors. Complement has been shown to be an important element in the innate immune response to systemic fungal infections; and several components of the fungal cell wall have been implicated in complement activation, e.g. β glucan, galactomannan or glucuronoxylomannan (for a recent review, see Speth et al. 2004; also see Chapter 11 in this volume). Although fungi are resistent to the membrane attack complex, complement factors can enhance the phagocytic clearance by opsonization and additionally attract and activate phagocytes. Specific binding of factor H and FHL-1, two regulatory components of the complement cascade, to the surface of *Can. albicans* may therefore provide an important mean for immune evasion (Meri et al. 2002).

The major components of the fungal cell wall, e.g. β(1–3) glucan, chitin and different kinds of mannans, are not found in humans and therefore constitute potential PAMPs (Bowman and Free 2006). In recent decades, several soluble or cell-bound lectins have been described which recognize fungal pathogens (Brown 2006). The family of collectins, for example, comprises several prominent lectin PRRs, like the mannose-binding lectin (MBL), the surfactant proteins A and D and the long pentraxin-3 (PTX3). Collectins are calcium-dependent (C-type) trimeric lectins which bind to surface carbohydrates of pathogenic fungi and enhance their clearance (van de Wetering et al. 2004). The collectins surfactant protein A and D are present in the lumen of the lung and they bind to the surface of fungal pathogens. Opsonization by surfactant proteins can have different effects: (a) it may enhance the phagocytic uptake, as it has been reported for *A. fumigatus* conidia (Madan et al. 1997), (b) it may have no effect on phagocytosis (Walenkamp et al. 1999) or (c) it may even hamper phagocytosis by the formation of large fungal aggregates, as has been reported for *Can. albicans* and *Cry. neoformans* (van de Wetering et al. 2004). PTX3 is a long pentraxin that directly binds to the surface of *A. fumigatus* conidia and is essentially required for protection against *A. fumigatus* infections in mice (Garlanda et al. 2002).

Cell-bound mannose receptors have been implicated in the phagocytosis of fungi (Newman and Holly 2001). More recent data, however, indicate that the human mannose receptor is not a professional phagocytic receptor, but rather a binding molecule which mediates the endocytic uptake of soluble ligands and not the phagocytic internalization of particulate ligands, like zymosan (Le Cabec et al. 2005).

More recently three additional C-type lectins have been implicated in anti-fungal immunity: the dendritic cell-specific intercellular adhesion molecule 3-grabbing non-integrin (DC-SIGN), the SIGN-related 1 (SIGNR1) and dectin-1. DC-SIGN is a type II C-type lectin that recognizes *A. fumigatus* conidia and triggers their phagocytic uptake by dendritic cells and macrophages. This interaction could be blocked by *A. fumigatus* galactomannan or mannan preparations derived from other fungi (Serrano-Gomez et al. 2004). SIGNR1 has been shown to be engaged in the non-opsonic recognition of fungal particles by macrophages, although it was found to be only poorly phagocytic (Taylor et al. 2004). Specific binding to mannans has been established for DC-SIGN and SIGNR1 (Mitchell et al. 2000), while the third novel lectin, Dectin-1, has been identified as the major surface receptor for β(1–3) glucan on dendritic cells and macrophages (Brown et al. 2002). Several studies demonstrated a pivotal role for this non-TLR PRR in the recognition and internalization of different fungi (Brown 2006). Recent data suggest that apart from β(1–3) glucan, dectin-1 also recognizes PTX3 opsonized particles (Diniz et al. 2004). Binding of PTX3 to zymosan or *Paracoccidioides brasiliensis*

yeast cells was shown to strongly enhance the dectin-1-dependent phagocytic uptake by mouse macrophages.

The importance of β(1–3) glucan as a particular fungal PAMP is underlined by recent data demonstrating a elaborate fungal immune evasion strategy. It has been known for some time that *Histoplasma capsulatum* strains or mutants lacking α(1–3) glucan have a reduced virulence (Klimpel et al. 1988; Marion et al. 2006). Rappleye and co-workers have now demonstrated that an outermost layer of α(1–3) glucan prevents recognition of the major cell wall constituent β(1–3) glucan by dectin-1 (Rappleye et al. 2007). This camouflage has been proposed as a determinant essentially defining the different pathogenic potentials of dimorphic fungal pathogens and opportunistic fungi. While a huge body of evidence supports the concept of dectin-1 being a crucial PRR for anti-fungal immunity, recent analysis of dectin-1 knock-out mice came to controversial results on the relevance of dectin-1 for certain fungal infections. Taylor and co-workers (2007) reported that dectin-1 deficient mice are susceptible to systemic *Can. albicans* infections accompanied by a reduced ability to recruit neutrophils and inflammatory monocytes to the site of infection. In contrast, Saijo et al. (2007) found that dectin-1 is not essential for clearance of *Can. albicans* infections, but is required for resistance against *Pneumocystis carinii*. The cytokine production during *Pneumocystis* infection was similar in dectin-1 knock-out and wild-type mice, but further analysis revealed that dectin-1-deficient macrophages showed a defective production of reactive oxygen species. Whether differences in the fungal strains or the infection protocols account for the contradictory results with respect to *Candida* infections remains to be determined. The earlier finding that deletion of CARD9, a downstream signalling molecule of dectin-1, leads to susceptibility to *Candida* infection (Gross et al. 2006) supports the concept of dectin-1 being required to mount an efficient anti-*Candida* immune response.

Recent research, starting with the results of Gantner et al. (2003), provided a large body of experimental evidence suggesting a tight functional interaction between dectin-1 and TLR2, which exemplifies a cross-talk between two structurally unrelated PRRs. Further collaborations between members of the TLR family and innate immune receptors have been described in the recent review by Mukhopadhyay et al. (2004). These data provide a first glimpse into the complex network of signalling receptors present on the surface of immune cells that drives and controls the immune response to microbial pathogens.

V. Relevance of Distinct TLRs in the Immune Responses to Different Fungi

The innate immune response is crucial for the control of fungal infections and the role of TLRs in anti-fungal immunity has been covered by several recent reviews (Levitz 2004; Netea et al. 2004; Roeder et al. 2004b; Romani 2004). Systemic fungal infections usually start either with the inhalation of fungal cells or from a persistent colonization of a mucosal or cutanous surface. During systemic spread, the fungus must be able to survive the attacks of immune cells and to breach epithelial or endothelial barriers. Survival in the infected tissue requires further adaptive abilities, e.g. the competition with the host for ferric iron or the evasion of immune defense mechanisms.

The pathogenic dimorphic fungi are exogenous pathogens that are usually taken up by inhalation and are capable of causing a systemic infection even in the immunocompetent host. The development of molecular biological tools for analysis of these pathogens and the availability of genomic information allowed the identification of virulence mechanisms employed by these pathogens to overcome the host pulmonary defenses (Rappleye and Goldman 2006). It appears likely that recognition by TLRs and other PRRs determines the outcome of infection. However, thus far only one report has been published on the interaction of a dimorphic fungus with TLRs. In this study, Viriyakosol and co-workers (2005) demonstrate that TLR2 plays an important role in the innate immune response to *Coccidioides posadasii*.

Our knowledge on the PRRs engaged in the defense of opportunistic fungal pathogens is much broader and the role of TLRs, especially TLR2 and TLR4, has recently been analyzed in much detail. Several studies have been published analyzing the role of TLRs in the immune response to *Can. albicans*, *A. fumigatus* and *Cry. neoformans*, and in less detail also to several other opportunistic fungi. It is generally accepted that a Th1 type immune

response is required for clearance of fungal infections and that TLRs play a major role in modulating the anti-fungal immune response (Romani 2004). However, as outlined below, there is still a debate on the relative importance of certain TLRs (and non-TLR receptors) for the recognition of different opportunistic fungi. This problem is related to the fact that the relevant fungal TLR ligands have not been precisely defined yet.

A. *Candida albicans*

Candida is a commensal microorganism that is not typically found in the environment, but colonizes the human gastrointestinal tract and in particular its mucosal surfaces. It therefore represents the prototype of an opportunistic fungal pathogen causing endogenous infections. Severe candidiasis requires a local or systemic impairment of the immune surveillance which results in either a local and superficial or a severe and systemic infection. *Can. albicans* can switch from a yeast-like morphotype to hyphal growth, a process which has been discussed as a major virulence trait. During the past decades molecular genetic studies have confirmed this hypothesis and further demonstrated that genes governing cellular morphology are co-regulated with genes encoding conventional virulence factors such as proteases and adhesins (Kumamoto and Vinces 2005). The transcriptional regulatory networks of *Can. albicans* thus ensure that virulence factors are produced in hyphae, but not in the yeast form. Hyphae are able to exert a mechanical force, aiding the penetration of epithelial and endothelial barriers, which is a prerequisite for an efficient systemic spread of infection. The tightly regulated hyphal morphogenesis therefore represents an integral part of the overall virulence strategy of *Can. albicans*.

Although most of the research activities are still focussed on *Can. albicans* as a model organism for an opportunistic fungal pathogen, other *Candida* species are also well known to cause severe infections in immunocompromised individuals. These strains are of increasing importance due to the fact that the incidence of non-albicans infections is rising, at least in certain parts of the world, and that certain non-albicans species have an enhanced resistance to commonly used antimycotics, like fluconazol (Perfect 2004). Interestingly, *Can. glabrata* has only a limited ability to form hyphae, but is still pathogenic (Csank and Haynes 2000). This is of special interest, because changes in the fungal surface which normally accompany the infection process have severe consequences for recognition by PRRs and consequently for setting up an appropriate immune response, as is discussed in more detail below.

The finding that the TLR adaptor protein MyD88 is required for the response of macrophages to *Can. albicans* (Marr et al. 2003) and for the mouse resistance to *Candida* infections (Bellocchio et al. 2004; Villamon et al. 2004c) has already suggested that certain TLRs play an important role during systemic candidiasis. Due to the availability of mice defective in either TLR2 or TLR4, research was mainly focussed on these two receptors. In the initial study, Netea and co-workers (2002) found that the absence of TLR4-mediated signals rendered mice highly susceptible to disseminated candidiasis, whereas in a following study, mice lacking TLR2 turned out to be less affected (Netea et al. 2004). This suggested that signalling through either of these two TLRs alone may govern the immune response in different directions. However, both kinds of infected mice showed normal levels of TNFα, indicating that lack of a single TLR was not sufficient to completely abrogate a proinflammatory immune response. Instead, the susceptibility of TLR4-deficient mice was attributed to an impaired production of the chemokines 'keratinocyte-derived chemokine' (KC) and the 'macrophage inflammatory protein-2' (MIP-2) and consequently an impaired recruitment of neutrophils (Netea et al. 2002), whereas the resistance of TLR2-deficient mice correlated with a reduced production of the anti-inflammatory cytokine IL-10 and a decrease in the regulatory T-cell population (Netea et al. 2004a). These findings suggested a protective role for TLR4, whereas a TLR2-dominated anti-inflammatory signalling seemed to play a deleterious role during systemic candidiasis. However, in human mononuclear cells blocking of TLR2, but not of TLR4, led to a significant inhibition of TNFα production (Netea et al. 2002), implicating TLR2, but not TLR4 in the pro-inflammatory response to *Candida* infections in humans. These data demonstrate that results obtained in the mouse system cannot automatically be transferred to the situation in humans. However, the importance of TLR4 in anti-*Candida* immune response in humans has recently been underlined by the finding that TLR4 Asp299Gly/Thr399Ile polymorphisms are a risk

factor for *Candida* bloodstream infections (Van der Graaf et al. 2006).

For a detailed understanding of the roles of certain PRRs in the immune response to certain microbial pathogens it is crucial to define the respective PAMPs that are recognized. In the case of fungal pathogens such PAMPs most likely reside within the cell wall, a complex and carbohydrate-rich structure (Bowman and Free 2006) that is unique to fungi and exposed to the surrounding environment. The outer layer of the *Candida* cell wall mainly consists of proteins which are glycosylated with either O- or N-linked carbohydrates, while β(1–3) glucan represents the major constituent of the underlying meshwork (Ruiz-Herrrera et al. 2006). Mannans have been demonstrated to induce the production of proinflammatory cytokines by TLR-4 signalling and might therefore represent a major fungal PAMP (Tada et al. 2002). Using a set of *Candida* mutant strains with defined defects in cell wall synthesis, Netea et al. (2006a) recently provided evidence that O-linked mannans are recognized by TLR4, whereas recognition of N-linked mannan is mediated by the mannose receptor. Mutants with defects in N-linked carbohydrate induced a severely impaired production of proinflammatory cytokines in human mononuclear cells and mouse macrophages. Similar, but less dramatic results were obtained with mutants harboring truncated O-linked mannans suggesting that the pro-inflammatory response to *Candida* can largely be attributed to TLR4- and mannose receptor-dependent signals. The residual mannan-independent proinflammatory cytokine production appears to be triggered by recognition of β(1–3) glucan through the dectin-1 receptor, a process which requires TLR2 for full efficiency (Netea et al. 2006a). This involvement of TLR2 in the dectin-1 mediated recognition of β(1–3) glucan provides an explanation for the reduced production of TNFα that was previously observed for human mononuclear cells treated with anti-TLR2 antibodies (Netea et al. 2002).

The study of Netea et al. (2006) is of special interest, as it identifies several ligands for PRRs and analyzes their relative contribution to an anti-*Candida* immune response. These data suggest that the use of mutants defective in defined structures or components of the cell wall provides a promising approach that is likely to gain detailed insights into the interplay between immune receptors and their PAMP counterparts in the cell wall. The evidence for a important or even dominant role of mannan-receptor- and dectin-1-dependent signals for cytokine production underlines the importance of non-TLR receptors in the immune response to *Candida*, but these data also point to the complexity of the signalling network which in the response to every single microorganism consists of a cross-talk and interplay of different Toll-like and non-Toll-like receptors. As already mentioned above, two papers have been published very recently describing the phenotype of dectin-1 knock-out mice. One report came to the conclusion that dectin-1 has a fundamental function in antifungal immunity, evidenced by the susceptibility of such mice to systemic *Can. albicans* infections (Taylor et al. 2007), whereas the other revealed no obvious role for dectin-1 in an anti-*Candida* immune response, but in the host defense against *Pneumocystis carinii* infections (Saijo et al. 2007). The latter study furthermore provided evidence that the production of inflammatory cytokines in response to zymosan is largely due to a MyD88- (and likely TLR-) dependent signalling, whereas dectin-1-mediated signals are of less importance. These data should remind us that zymosan is not a homogenous β(1–3) glucan particle, as sometimes supposed, but a complex structure containing other components, including mannans, other glucans and chitins (Di Carlo et al. 1958).

While it is generally assumed that recognition via TLR4 favors the production of pro-inflammatory cytokines, the situation is less clear for TLR2. Like TLR4, this receptor is able to trigger the production of pro-inflammatory cytokines (Hirschfeld et al. 2001) and stimulation of an inflammatory response by TLR2 is especially prominent in response to $Gram^+$ bacteria (Takeuchi et al. 1999) and when TLR2 recognizes fungal PAMPs in concert with dectin-1 (Gantner et al. 2003). However under certain conditions TLR2 signalling can also provide a strong anti-inflammatory stimulus (Netea et al. 2004b). Recent data provided evidence that *Candida* yeasts and blastoconidia are recognized by TLR4, while hyphae are not (d'Ostiani et al. 2000; Van der Graaf et al. 2005); and it has been concluded that the resulting inability of hyphae to stimulate IL-12 or IFNγ production represents an immune evasion strategy (Netea et al. 2004b). Lack of TLR4-mediated signals during invasive hyphal growth may shift the balance towards a TLR2-mediated and more anti-inflammatory IL-10 dominated signalling.

Such TLR2-dominated signalling furthermore seems to stimulate the clonal expansion of CD4+ CD25+ regulatory T-cells (Treg; Netea et al. 2004), which are well known to down-regulate an inflammatory response. While this is a crucial step in bringing back a successful immune response to homeostasis, it can be deleterious during a still unsolved infection. This assumption is confirmed by the finding that depletion of Tregs improved the resistance of mice to systemic candidiasis (Netea et al. 2004). Overwhelming septic immune responses are typically found in systemic bacterial infections while they are less common in patients suffering from systemic mycoses. This may result from differences in the regulation of the immune responses to bacterial and fungal pathogens. It might be possible that, in comparison to bacterial infections, there is not such an urgent need for a strong anti-inflammatory signalling after clearance of fungal pathogens, whereas the limited pro-inflammatory signals induced by many fungal pathogens might be essentially required to establish a successful immune response.

From the data summarized so far a picture emerges for the immune response to systemic candidiasis that seems to be consistent, but there are still several points that need to be addressed in more detail. One question is whether or not surface exposed short O-linked mannans are present on the hyphal surface or whether they are shielded from TLR4 by certain, yet unknown surface structures. There are data suggesting that mannoproteins exist in the cell wall of *Candida* yeasts and hyphae, which contain O-linked mannans and might in principle be recognized by TLR4. Along this line are recent data demonstrating binding of the soluble mannan-binding lectin to *Candida* yeasts and hyphae in the infected tissue (Lillegard et al. 2006).

The data of Netea and co-workers provide strong evidence that TLR4 is required for protection to candidiasis, while TLR2 signalling is dispensable or under certain conditions even harmful for *Candida*-infected mice. However, the relative importance and function of TLR2 and TLR4 in the immune response to systemic *Candida* infections is still a matter of discussion. Villamón and co-workers (2004a) found that TLR2-deficient mice are more susceptible to candidiasis and Murciano et al. (2006) reported that TLR4 is dispensable in a mouse model of a hematogenously disseminated *Candida* infection. Consistent data obtained from in vitro experiments provided evidence that the production of proinflammatory cytokines to *Candida* yeasts and hyphae is impaired in TLR2-, but not in TLR4-deficient macrophages (Villamón et al. 2004a; Gil and Golzalbo 2006). Taken together, these data emphasize the importance of TLR2, but not TLR4, for the clearance of *Candida* infections. Additionally, it has been shown that the low molecular weight phospholipomannan of *Can. albicans* binds to macrophages and induces the production of TNFα in a TLR2-dependent manner (Jouault et al. 2003), again supporting the concept of TLR2 as a major factor involved in the inflammatory response to *Candida*.

In a study by Bellocchio and co-workers (2004) mice defective in either MyD88, TLR2, TLR4, TLR9 or IL-1 receptor were infected with yeasts or hyphae of *Can. albicans*. All mice infected intravenously with the highly pathogenic hyphal form succumbed to infection, whereas TLR4- and TLR9-deficient mice survived significantly longer. In contrast, only MyD88- and IL-1 receptor-deficient mice died after intravenous infection of the less virulent *Can. albicans* yeasts. These data suggest that neither TLR4, nor TLR2 is essentially required in this mouse model of systemic candidiasis and consistently, neutrophils from TLR2- and TLR4-deficient mice were not impaired in their antifungal activity to *Candida* yeasts and hyphae (Bellochio et al. 2004).

Mice having survived a primary infection with *Candida* yeasts were also re-infected with a normally lethal dose of *Candida* hyphae. While wild-type and TLR9-deficient mice were protected and survived this challenge, most TLR2- and TLR4-deficient mice died, suggesting a pivotal role for both receptors in the formation of an immunological memory (Bellocchio et al. 2004). However, using a similar experimental setup, a more recent study came to the conclusion that TLR2 is dispensable for protection of mice immunized with a sub-lethal challenge of *Can. albicans* (Villamón et al. 2004b). The influence of anti-mycotica treatment on the immunological recognition of *Can. albicans* was analyzed in a study by Roeder et al. (2004a). Evidence was reported that anti-mycotica treatment has a strong impact on the relative importance of TLR2- and TLR4-signalling. This might result from differential surface exposition of certain cell wall components in anti-mycotica treated versus untreated *Candida* cells.

In summary, the data published so far on the relative importance of TLR2 and TLR4 in the mouse and human immune response to *Can. albicans* infections are not fully consistent. The reasons for the observed discrepancies can be multifaceted, but it is likely that differences in the strains used contribute to the controversial results. Subtle differences in the organization and regulation of *Candida* cell wall components might occur and have to be taken into account. Comparative studies using different strains might therefore provide new and important insights and could help to solve the current discrepancies.

B. *Aspergillus fumigatus*

Aspergillus fumigatus is a saprophytic fungus commonly found in the soil and on decaying organic matter. The ubiquitous presence of this mold in the environment results from the efficient distribution of its conidia through the air. These spores are taken up by inhalation and reach the alveoli of the lung due to their small size. Although humans inhale large numbers of *A. fumigatus* conidia every day, life-threatening, systemic *Aspergillus* infections are rare and restricted to severely immunocompromised patients, a fact which reflects the efficient surveillance of the lung by the innate immune system. Interestingly, recent findings nevertheless suggest that sub-clinical *A. fumigatus* infections may occur and precede hospitalization and immune suppression (Sarfati et al. 2006). Latent *Aspergillus* infections may therefore represent an underestimated risk factor for immunocompromised patients vulnerable to develop an invasive aspergillosis.

Alveolar macrophages are primarily responsible for the elimination of inhaled conidia, as they are able to track down and kill them by phagocytosis. Degradation of the ingested spores in the phagolysosome and the production of reactive oxygen species are means that have been implicated in the killing of engulfed conidia (Ibrahim-Granet et al. 2003; Philippe et al. 2003). In contrast to macrophages, neutrophils have to be recruited to the site of infection. They form a second line of defense and possess the unique ability to attack and kill not only conidia and germtubes, but also hyphae, which may escape phagocytosis due to their size. Neutrophils can recognize such elongated fungal elements by PRRs (Bellocchio et al. 2004b) and kill them by the degranulation and release of aggressive molecules, e.g. reactive oxygen species (Schaffer et al. 1982). Natural killer (NK) cells represent another type of immune cells that can be recruited to the *Aspergillus*-infected lung by the release of the 'chemokine ligand monocyte chemotactic protein-1', also designated 'chemokine (C-C motif) ligand-2' (MCP-1/CCL2). Recent data by Morrison et al. (2003) demonstrate that NK cells represent a previously unrecognized and critical element in the early host defense mechanism during invasive aspergillosis and contribute to the residual anti-fungal protection of neutropenic mice. Macrophages and dendritic cells can both engulf and kill fungal cells and can additionally communicate with the adaptive immune system. In this respect, dendritic cells, are of pivotal importance as they largely determine whether a protective pro-inflammatory Th1-like response is induced or not (Romani et al. 2002).

Like *Can. albicans*, *A. fumigatus* undergoes a morphological transition during infection, leading from small and metabolic inactive conidia to elongated, growing hyphae. Further intermediate morphotypes, like swollen conidia or germlings, also differ in their surface properties, and even hyphae expose certain molecules or structures only in restricted parts of their surface (Momany et al. 2004). This results in a complex pattern of biochemical distinct surface structures that changes substantially during infection, being mainly proteinaceous in resting conidia (Paris et al. 2003), but rich in carbohydrates in hyphae, germlings and swollen conidia (Bernard and Latge 2001). Consequently, the relative importance of certain PRRs may differ for the various morphotypes and the immune response to *A. fumigatus* therefore relies on a dynamic pattern of PRR-PAMP interactions.

While *Candida* has co-evolved with its human host over a long time, the situation is different for *A. fumigatus*. This mold is primarily an environmental and saprophytic organism. Severe systemic infections are rare and merely caused by the increased number of susceptible human hosts due to recent progress in modern medicine. It therefore appears unlikely that *Aspergillus* infections in humans had a substantial impact on the evolution of this fungus. It is, nevertheless, striking that from more than 200 *Aspergillus* species, only *A. fumigatus* is responsible for over 90% of all cases of invasive aspergillosis (Latge 1999).

This indicates that *A. fumigatus* indeed must have some distinct properties that facilitate a pathogenic life in the immunocompromised human host. The recent availability of genome sequences for *A. fumigatus* (Nierman et al. 2005), the non-pathogenic *A. oryzae* and *A. nidulans*, as well as fungal pathogens, like *Can. albicans* and *Cry. neoformans* enabled a search for characteristic traits of fungal virulence (Galagan et al. 2005). *A. fumigatus* seems to lack specific pathogenic elements, but is instead well equipped with numerous efflux pumps, an elaborate apparatus for environmental sensing, mechanisms and molecules to counteract reactive oxidants and an ability to retrieve and exploit a broad spectrum of nutrients, which, in combination enable this saprotrophic mold to survive in the unfriendly environment of the human host (Tekaia and Latge 2005).

A better understanding of the mechanisms that lead to progression of an *A. fumigatus* infection in the immunosuppressed host is clearly an urgent task. An important step in this direction is the recent attempts to unravel the interactions between the different morphotypes of this mold and the immune cells of the immunocompetent and immunocompromised host. In two early reports, Taramelli et al. (1996) and Grazziutti et al. (1997) found that *A. fumigatus* conidia and hyphae induce an inflammatory cytokine response in human and murine macrophages. A first attempt to identify the underlying PRR-PAMP interactions was performed by Wang et al. (2001). Using human adherent monocytes and blocking monoclonal antibodies, those authors showed that *A. fumigatus* hyphae trigger an inflammatory response through TLR4-, but not TLR2-signalling. This process was shown to be dependent on CD14, a protein that is known to be engaged in the TLR4-mediated recognition of LPS (Chow et al. 1999).

In the following years, several studies analyzed the response of macrophages derived from either TLR2- and/or TLR4-deficient mice to *A. fumigatus* hyphae and conidia. The published data are in part controversial and range from a specific requirement for TLR2, but not TLR4 (Mambula et al. 2002), a requirement for TLR4 and to a lesser extent for TLR2 (Meier et al. 2003), to a mainly TLR4-dependent response to conidia, but not to hyphae (Netea et al. 2003). The latter data suggested an immune evasion strategy of *A. fumigatus* hyphae similar to that proposed for *Candida* (Netea et al. 2004b).

While the relative importance of TLR2 and TLR4 has been analyzed in some detail, only one study has been published so far that attempted to define the relative importance of the other members of the human TLR family. From a set of human HEK293 cells transfected with any of the ten human TLR genes only those expressing TLR2 or TLR4 showed a significant response to *A. fumigatus* (Meier et al. 2003). Experiments with transfected cells are commonly performed as they provide a mean to study the relevance of single receptors. However, cells used for transfection are usually not part of an anti-fungal immune response and care has to be taken in the discussion of the resulting data. Transfected cells likely provide an appropriate model for many TLRs, but might be less suitable to study the relevance of intracellular TLRs, which recognize PAMPs that have to be released from the respective microorganisms. TLR9 recognizes non-methylated CpG-motifs which are commonly found in bacterial, but not in human DNA. TLR9 might theoretically be able to recognize also *Aspergillus* DNA, which seems to lack methylation of CpG motifs (Antequera et al. 1984). However recognition of *Aspergillus* CpG motifs by TLR9 would require fungal lysis in the phagosome of professional phagocytes, which is unlikely to occur in HEK293 cells. In the transfection experiments mentioned above, this problem could have been bypassed by the use of hyphal fragments, which may have released sufficient DNA, but additional experiments are clearly required to rule out a potential involvement of TLR9 in the recognition of *A. fumigatus*.

Evidence arguing against an important role of most TLRs came from experiments with mouse macrophages that were defective in both TLR2- and TLR4-signalling. These phagocytes were not able to respond to *A. fumigatus* using different read-out parameters (Meier et al. 2003) suggesting that other TLRs, apart from TLR2 and TLR4, are of only minor importance for recognition of *A. fumigatus* by isolated mouse macrophages.

According to a recent study, not only intact *A. fumigatus* cells, but also released *A. fumigatus* molecules have the capacity to activate innate immune cells through TLR2- and TLR4-signalling (Braedel et al. 2004). This finding opens up the interesting perspective to isolate and identify soluble *Aspergillus* PAMPs that are released from the fungal cell wall during growth.

As summarized above, the relative importance of TLR2 and TLR4 for the macrophage-mediated response to different *A. fumigatus* morphotypes is still a matter of discussion. The numerous reasons which may account for the sometimes controversial results obtained in the different studies have been discussed recently (Luther and Ebel 2006).

Infection experiments in mice defective in certain PRRs provide an important proof to test the relevance of certain receptors for the clearance of infection. So far two studies have been undertaken to analyze the role of TLR2 and TLR4 in a mouse model of systemic aspergillosis. Both sets of data indicate that immunocompetent TLR4- and TLR2-deficient mice showed a normal resistance to *A. fumigatus* infections, whereas an immunosuppressive treatment rendered them susceptible to infection (Bellocchio et al. 2004a; Balloy et al. 2005). This suggests that, in the immunocompetent host, resident phagocytes in the lung are able to clear the infection without any need for a recruitment of other effector cells, which likely requires a TLR-dependent signalling. After immunosuppression the residual capacities of the phagocytes has to be well orchestrated and coordinated to achieve protection and, in this context, TLR2 and TLR4 are obviously required. In line, a recent study with human patients revealed that certain polymorphisms in TLR1 and TLR6 are associated with a higher risk to develop an invasive aspergillosis after allogenic stem cell transplantation (Kesh et al. 2005). Since TLR1 and TLR6 are well known to form functional heterodimers with TLR2 (Ozinsky et al. 2000) these data also point to an important role of TLR2 in the anti-*Aspergillus* immune response.

Recently, the role of dectin-1 in the immune response to *A. fumigatus* was analyzed in some detail. Several studies came to the conclusion that recognition of β(1–3) glucan by dectin-1 is essentially required to stimulate a proinflammatory cytokine response and to trigger the recruitment of neutrophils to the site of infection. All studies revealed that the proinflammatory response to germinating conidia is much more pronounced than to resting conidia, which correlated well with differences in the surface display of β(1–3) glucan in both kinds of spores (Hohl et al. 2005; Steele et al. 2005; Gersuk et al. 2006). Consistent with the previous finding that dectin-1 can trigger phagocytosis of fungal particles (Herre et al. 2004) it was shown that dectin-1 is also engaged in phagocytosis of both *A. fumigatus* germlings and conidia (Gersuk et al. 2006; Luther et al. 2007).

Evidence for a collaborative induction of inflammatory responses by dectin-1 and TLR2 came originally from a study by Gantner et al. (2003) focussing on the response of macrophages to β(1–3) glucan-rich zymosan particles. Subsequent studies provided supportive evidence for a cross-talk between TLR2 and dectin-1, e.g. in the immune response to the bimorphic pathogenic fungus *Coccidioides posadasii* (Viriyakosol et al. 2005); and this has recently been confirmed for the immune response to *A. fumigatus* (Hohl et al. 2005; Gersuk et al. 2006). Evidence from other experimental systems already suggested that TLR signalling may also modulate phagocytosis (Underhill and Gantner 2004). For *A. fumigatus* TLR2-signalling, but not TLR4-signalling, was found to positively modulate the phagocytic uptake of conidia by macrophages (Luther et al. 2007), which suggests that the cross-talk between TLR2 and dectin-1 is not restricted to the production of proinflammatory cytokines.

C. *Cryptococcus neoformans*

Cryptococcus neoformans is the etiological agent of cryptococcosis, a disease that is a major cause for meningoencephalitis in immunocompromised individuals and is of particular importance in patients suffering from AIDS. The most striking feature of this opportunistic yeast is its capsule, which represents a major virulence trait evidenced by the fact that non-capsulated strains are non-pathogenic. The structure, assembly and regulation of this important anti-phagocytic factor have been recently reviewed by Bose et al. (2003). The capsule masks potential ligands in the cell wall and consequently, unopsonized *Cry. neoformans* are poorly phagocytosed. Glucuronoxylomannan (GXM) represents the major constituent of the capsule and the density of the GXM matrix was found to be much higher in capsules from cells isolated from infected tissue, than from yeasts harvested after in vitro growth (Gates et al. 2004). GXM is shedded during infection and detectable in blood, cerebrospinal fluid and the infected tissue. Furthermore it exhibits potent immunosuppressive properties (for a recent review, see Monari et al. 2006). Ligation of GXM to TLR4 on macrophages was shown to mediate a fast and long-lasting

up-regulation of the Fas ligand, which enables GXM-loaded macrophages to induce apoptosis in Fas-expressing T-cells through activation of caspase-8 (Monari et al. 2005; Pericolini et al. 2006). Inhibition of neutrophil recruitment is another mean by which TLR4 signalling can impair the anti-*Cryptococcus* immune response. GXM has been shown to prevent the rolling and fixed binding of neutrophils to the endothelium, steps which are crucially required for the migration of neutrophils out of the vessel and into the tissue. This effect of GXM can be blocked using monoclonal antibodies directed to TLR4 or CD14 (Ellerbroek et al. 2004a), and O-acetylation of the GXM was shown to be essential for this interference with neutrophil migration (Ellerbroek et al. 2004b). In conclusion, these data suggest that 6-O-acetylated mannose might be a novel fungal TLR4 ligand.

In vitro experiments using transfected CHO cells demonstrated enhanced binding of GXM to cells expressing TLR2, TLR4 and/or CD14, whereas an activation of NF-kB was restricted to TLR4/CD14 expressing cells. Incubation of human peripheral blood monocytes (PBMCs) with GXM also led to the activation of NF-kB, but surprisingly not to a release of TNFα (Shoham et al. 2001), suggesting that GXM triggers several and interfering signalling events. Inhibition of an inflammatory response to LPS by GXM was already observed more than ten years ago by Vecchiarelli and co-workers (1996); and this effect was later attributed to the GXM-triggered secretion of the anti-inflammatory cytokine IL-10 (Vecchiarelli et al. 1996).

The data presently available emphasize an immunomodulatory role for released GXM, which provides a strong anti-inflammatory stimulus. The impact of TLR signalling during *Cryptococcus* infection has so far been addressed in two studies using murine models of cryptococcosis. In the first report, Yauch and co-workers (2004) found that, after intranasal infection, mice lacking TLR2 or CD14 were more susceptible than the corresponding wild-type mice, whereas no difference was observed after an intravenous infection route. Lack of TLR4 had no consequences for the outcome of infection, while an important role in the immune response to *Cryptococcus* was attributed to MyD88, an adaptor molecule essential for the signalling of IL1 receptor and most members of the TLR family (Yauch et al. 2004). An important role for MyD88 and TLR2 was confirmed in a study of Biondo et al. (2005), who also found that TLR4 is dispensable in this context. A more recent study came to the conclusion that TLR2 and TLR4 are only of limited importance for resistance of mice to *Cry. neoformans* infection (Nakamura et al. 2006) and this was supported by the finding that HEK293 cells expressing either TLR2/dectin-1 or TLR4/MD2/CD14 showed no activation of NF-kB in response to *Cryptococcus* (Nakamura et al. 2006). In summary, all published data suggest that TLR4-signalling is not protective during cryptococcosis, but that GXM-triggered TLR4-signalling might in fact hamper an efficient clearance of infection. At least two reports suggest a protective role for TLR2 and MyD88 in the anti-*Cryptococcus* immune response. Additional attempts are clearly required to define the role of TLR2 in the defeat of *Cry. neoformans* infections.

D. TLR-Mediated Recognition of Other Pathogenic Fungi

Macrophages respond to the infectious spherule form of the bimorphic pathogenic fungus *Coccidioides posadasii*, the causative agent of the coccidioidomycosis, in a TLR2-, MyD88- and dectin-1-dependent manner, but this inflammatory response appears to be independent of TLR4 (Viriyakosol et al. 2005).

Pseudallescheria boydii with its asexual form, *Scedosporium apiospermum*, is now recognized as an important emerging opportunistic pathogen causing invasive mycosis in immunocompromised patients (O'Bryan 2005). A linear 4-linked α-D-glucan from the conidial surface of *P. boydii* has been isolated and analyzed. This soluble α-D-glucan inhibits conidial phagocytosis and triggers the secretion of cytokines in a TLR2-, CD14- and MyD88-dependent manner (Bittencourt et al. 2006).

The lipophilic yeast *Malassezia furfur* is the causative agent of pityriasis versicolor (Crespo-Erchiga and Florencio 2006) a common disorder of the skin, which is characterized by scaly hypo- or hyperpigmented lesions. *M. furfur* was shown to induce expression of TLR2 and MyD88 in human keratinocytes and IL-8 expression and secretion was observed to depend on TLR2 (Baroni et al. 2006).

Pneumocystis jirovecii is a common cause of pneumonia in immunocompromised patients and substantially contributes to the morbidity and

the mortality of patients suffering from AIDS or malignancies (Gigliotti and Wright 2005). β(1–3) Glucan-rich *Pneumocystis* cell wall fractions have been isolated and analyzed (Lebron et al. 2003). Pneumocystis β glucan trigger an activation of NF-kB and the production of TNFα in macrophages in a MyD88-dependent manner, whereas no evidence has been obtained for the involvement of TLR4 (Lebron et al. 2003).

Fungi are well known to produce a large spectrum of allergens (Horner et al. 1995) and TLRs are likely to play an important role in recognition of these molecules. A Th2-dominated immune response is required for the development of asthma and TLRs have the capacity to polarize the T helper cell bias of an adaptive immune response into opposing directions. One proposed explanation for the increased prevalence rates of allergic diseases in the developed countries is the so-called 'hygiene hypothesis' (Bach 2002), which hypothesized that infection in early childhood acquired through unhygienic contact helps to prevent the development of allergic disease, whereas the decreased exposure to microbes caused by modern public health practices is supposed to lead to deficiencies in an important source of immune education (Horner 2006a). Several recent epidemiologic surveys showing an inverse relationship between the frequency of infectious disease and the incidence of allergic diseases lend support to this hypothesis, but the underlying mechanisms are still poorly understood. So far, only a few attempts have been undertaken to define the role of TLR polymorphisms for the development of asthmatic diseases and the functional role of TLRs for the reaction of airways to inhaled allergens is still largely unexplored (Kauffman 2006). Nevertheless, pharmacologic interventions that target TLR-signalling are supposed to possess an important clinical potential for the prevention and treatment of allergic diseases.

VI. Summary and Outlook

A large body of evidence suggests that TLRs are important PRRs controlling innate immune responses to living fungal pathogens and non-infectious but immunostimulatory fungal materials. While the principal importance of TLRs for the innate immunity is generally accepted, there is a controversial discussion about the precise role of distinct TLRs in the immune response to different pathogenic fungi. There are many factors that might contribute to this discontenting situation, including: (a) the use of different fungal strains and morphotypes, (b) the use of different kinds of immune cells and (c) differences in the experimental design. Certainly the sometimes controversial results also reflect the difficulties in analyzing the complex interplay between an array of often poorly defined ligands on the one hand, and a network of signalling receptors on the other, some of them being not well characterized.

Research on the relevance of TLRs for fungal infections is severely hampered by the fact that our knowledge of the respective PAMPs is still in its infancy. However, some progress has been made recently and several fungal glycostructures have been implicated as ligands for either TLR2 or TLR4. From the data summarized in Table 12.2 it is striking that certain types of mannans seem to be of special importance. However, even subtle biochemical modifications might have a strong impact on the potential of the respective structure to trigger a TLR-dependent signalling and therefore even slight impurities or contaminations of the mannans under investigation have to be excluded. Since glycostructures isolated from a fungal cell wall are notorious for being heterogeneous and difficult to analyze in detail, a combined effort of (glyco-)biochemistry and immunology is required to identify the relevant structures.

The finding that TLRs control immune responses to fungal pathogens and determine their T helper cell bias raised the possibility to target such receptors for the manipulation of an immune

Table 12.2. Putative fungal pathogen-associated molecular patterns (PAMPs) recognized by Toll-like receptors (TLR)

PAMP	Fungal pathogen	TLR	Reference
Phospho-lipomannan	*Candida albicans*	TLR2	Jouault et al. (2003)
Linear α(1–4) glucan	*Pseudallescheria boydii*	TLR2	Bittencourt et al. (2006)
Mannan	*Can. albicans, Saccharomyces cerevisiae*	TLR4	Tada et al. (2002)
Glucuronoxylomannan	*Cryptococcus neoformans*	TLR4	Shoham et al. (2001)
O-Linked mannan	*Can. albicans*	TLR4	Netea et al. (2006a)

response. Pharmacologic interventions may be aimed at shifting the T helper cell bias towards either a protective Th1-like pro-inflammatory or an anti-inflammatory response. The application of anti-TLR2 monoclonal antibodies was shown to prevent a septic shock in mice challenged with lipopeptides (Meng et al. 2004); and humanized antibodies may provide excellent tools to block or reduce a hyper-activation of innate immune responses. Modulation of an antifungal immune response by TLR ligands has also been discussed, e.g. in the context of asthma (Horner 2006b). We are now facing novel perspectives for treatment of infections and allergic or autoimmune diseases, but we have to keep in mind that our knowledge of the underlying mechanisms is still very limited. The modulation of an immune response by the use of an array of defined TLR ligands is certainly an exiting perspective, but any manipulation of a TLR-mediated immune response may also have deleterious and life-threatening consequences for the patients (Ishii et al. 2006). Any attempt to modulate an existing immune response therefore needs to be accurately balanced and restricted to the safe side of its potential use.

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