Role of the dual interaction of fungal pathogens with pattern recognition receptors in the activation and modulation of host defence

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ABSTRACT

Recognition of pathogen-associated molecular patterns (PAMPs) of microorganisms by pathogen recognition receptors induces signals responsible for the activation of genes important for an effective host defence, especially those of pro-inflammatory cytokines. Toll-like receptors (TLRs) and lectin-like receptors are the most important classes of pattern-recognition receptors. In addition to their effects on the activation of host defence, recent studies suggest that pathogenic fungi can modulate or interfere with the pattern recognition mechanisms of innate immunity, and can use pattern recognition receptors as mechanisms of escape from host defence. Two major recognition receptor-mediated escape mechanisms have been identified during infection with fungal pathogens: immunosuppression induced by activation of certain pattern recognition receptors, especially induction of IL-10 release through TLR2; and the blockade of TLR recognition by antigen modification during the germination of yeasts into hyphae. Thus, signals mediated by recognition receptors are not only beneficial to the host, but in certain situations can be used by pathogenic fungi to escape immune recognition and promote infection.

Keywords: Fungi, host defence mechanisms, immune evasion, review, Th1/Th2 balance, Toll-like receptors

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INTRODUCTION

Within minutes of the invasion of the host by a pathogenic microorganism, the innate immune system is activated and coordinates the host defence during the initial hours and days of the infection. Although the innate immune system is very effective in dealing with the vast majority of invading pathogens, it has been believed for many years to be non-specific and non-selective, with specificity being conferred only by the secondary activation of acquired immunity mediated by T- and B-lymphocytes. This dogma of the non-selective nature of the innate immune response and, in particular, the presumed non-specific recognition of microorganisms by phagocytic cells, has been challenged by the recent discovery of classes of receptors which are able to recognise specific molecular patterns of pathogenic microorganisms, called pattern-recognition receptors (PRRs). The most important classes of PRR are the Toll-like receptors (TLRs) and the lectin-like receptors. TLRs recognise a wide variety of microbial structures, including lipopolysaccharide (LPS) of Gram-negative bacteria (TLR4), lipoteichoic acid and lipoproteins of Gram-positive bacteria (TLR2), bacterial DNA (TLR9), and viral RNA (TLR3, TLR7 and TLR8) [1]. Lectin-like receptors are PRRs specialised in the recognition of polysaccharide chains of bacterial pathogens, including the mannans and glucans of pathogenic fungi [2]. Recognition of pathogens by PRRs leads to release of pro-inflammatory cytokines and activation of antibacterial mechanisms, and modulates the initiation of acquired immunity by the activation of dendritic cells.

PRRs MEDIATE PROTECTION AGAINST FUNGAL PATHOGENS

Several classes of recognition receptors mediate recognition of fungal pathogens. The structure of
the fungal cell-wall, which is composed mainly of carbohydrate chains such as mannans and glucans, meant that the first group of receptors discovered to mediate fungal recognition were the lectin receptors. Macrophage mannose receptors recognise fungal mannans and mediate recognition and phagocytosis of Candida spp. by macrophages [3], while another lectin-like receptor, DC-SIGN, participates in the phagocytosis of Candida spp. and Aspergillus spp. by dendritic cells [4]. In addition, the β-glucans of fungi are recognised by several receptors, such as complement receptor-3 [5], and the specific receptor dectin-1 [6]. However, the pathways by which leukocytes activated many of their antifungal mechanisms remained elusive until the discovery of TLRs.

The first suggestion of a role for TLRs in antifungal host defence was provided by the extreme susceptibility of Toll-deficient Drosophila to aspergillosis [7]. Shortly after the discovery of the human homologues of Toll, it became apparent that TLR2/TLR6 heterodimers recognise zymosan, a structure derived from Saccharomyces cerevisiae [8,9]. The cell-wall structure of Saccharomyces resembles closely that of the fungal pathogen Candida albicans, which prompted an investigation of the role of TLRs in the host defense against disseminated candidiasis. In the first description of the role of TLRs in a fungal infection, it was demonstrated that the absence of TLR4-mediated signals resulted in an increased susceptibility to disseminated candidiasis in TLR4-defective mice [10]. This effect was mediated through decreased release of the chemokines termed keratinocyte-derived chemokine (KC) and macrophage inflammatory protein (MIP)-2, and impaired recruitment of neutrophils at the site of infection. It has also been reported that the mannann components of the Candida cell-wall are the structures recognised by TLR4, a process which also requires CD14 [11].

Since these initial observations, further studies have confirmed the important role of TLRs in the recognition of C. albicans and anti-candidal host defence. The global role of TLRs in the host defence against disseminated candidiasis was demonstrated by the increased susceptibility of MyD88−/− mice, an intracellular adaptor molecule of the TIR domain, to C. albicans infection [12]. MyD88 was also involved in mediation of cytokine synthesis by C. albicans, as well as phagocytosis and killing of the yeast [12,13]. An important mechanism for the recognition of Candida and Saccharomyces spp. was demonstrated by two independent studies showing collaborative recognition of fungal β-glucan by TLR2 and the lectin-like receptor dectin-1 [14,15]. Experimental models of disseminated candidiasis infection in TLR2−/− mice have also shown modulatory effects of TLR2 on host defence [16–18]. In contrast, TLR9 appeared to be less important for host defence against C. albicans [18].

An important role of TLRs has also been demonstrated in the recognition of Aspergillus fumigatus. Invasive aspergillosis is a life-threatening infection which occurs predominantly in immunocompromised patients. As the number of immunocompromised patients has increased, A. fumigatus has become the second most common opportunistic fungal infection [19]. The involvement of TLRs in the recognition of Aspergillus was suggested for the first time by Wang et al. [20], who proposed TLR4, but not TLR2, as a receptor for Aspergillus hyphae. Subsequent studies have supported the hypothesis of TLR involvement in the recognition of Aspergillus by showing the important role of MyD88 in Aspergillus-induced cytokine production [18]. However, an exclusive role for TLR4 in the recognition of Aspergillus hyphae was not confirmed. In contrast, both TLR2 and TLR4 have been demonstrated to be important for recognition of A. fumigatus [21–24] and Aspergillus niger [23]. Similarly, TLR2 and TLR4 are crucial for neutrophil stimulation in aspergillosis [25]. The concept that TLR4-mediated pro-inflammatory effects are protective against invasive aspergillosis is supported by data showing increased susceptibility of TLR4−/− mice to A. fumigatus infection [18].

Significantly less is known concerning the interaction of the third major human fungal pathogen, Cryptococcus neoformans, with TLRs. Binding of C. neoformans glucuronoxylomannan to TLR2 and TLR4 has been reported, followed by NF-κB translocation in the case of interaction with TLR4, but not TLR2 [26]. However, despite induction of NF-κB activation, glucuronoxylomannan interaction with TLR4 failed to result in the release of pro-inflammatory cytokines [26]. More recently it has been shown that MyD88 and TLR2, but not TLR4, are required for host defence against infection with C. neoformans [27,28].
PRO- AND ANTI-INFLAMMATORY SIGNALS DURING INFECTION

Following activation of the innate immune system, strong pro-inflammatory signals are generated, inducing inflammation and activation of the host defence. After elimination of the invading microorganisms, subsequent anti-inflammatory signals are responsible for the resolution of inflammation [29]. These signals are crucial, not only for the return of the immune system to homeostatic balance, but also for the protection of the host against the deleterious effects of overwhelming inflammation and for subsequent tissue repair. Probably the best known example of an out-of-control inflammatory reaction during infection is the sepsis syndrome, in which generalised inflammation, induced by overproduction of cytokines, leads to hypotension, intravascular coagulation and multiple organ failure, resulting ultimately in death [30].

TLR-signals are involved in both the primary induction of inflammation and the secondary activation of anti-inflammatory mechanisms (Fig. 1). TLRs are known to induce release of anti-inflammatory cytokines, e.g., interleukin (IL)-10, IL-4, IL-5 and IL-13 [31,32], and TLR2- and TLR4-mediated signals have been shown to mediate generation of down-modulating T-regulatory cells [16,33]. In line with this concept, the absence of TLR2 or TLR4 results in increased mortality following overwhelming inflammation in certain experimental models, including those of pneumococcal meningitis [34] or *Bordetella pertussis* infection [35]. However, while TLR-mediated anti-inflammatory signals are beneficial following the elimination of the pathogens, they can induce dangerous immunosuppressive mechanisms if activated too early during a severe infection, as described below.

**FUNGAL PATHOGENS CAN USE PRRs AS ESCAPE MECHANISMS**

Recent studies of PRR biology have shown that, while innate recognition is crucial for an efficient immune response, certain fungal pathogens use PRR-based strategies to evade host defences. It has been demonstrated that TLR2 ligation can induce pro-inflammatory cytokines, but that this effect is weaker than that mediated by TLR4 [36]. In contrast, TLR2 signals are strong mediators of anti-inflammatory effects. The TLR2-induced immunosuppression is either an exaggeration or a premature activation of the normal anti-inflammatory effects of TLR stimulation, necessary during the recovery phase of the infection for reversing inflammation. The first study investigating the differential effects of TLR2 and TLR4 stimulation on dendritic cells reported the failure of TLR2 ligands to induce release of IL-12 and IFNγ, producing conditions favourable for a Th2-type response [31], and this was supported by additional studies [32,37,38]. Investigations of the molecular mechanism behind this phenomenon showed that engagement of TLR2/TLR1 heterodimers by the bacterial lipopeptide Pam3Cys resulted in stabilisation of the transcription factor c-Fos, a suppressor of IL-12, and that this resulted in the Th2 bias [32].

These in-vitro data have been accompanied by in-vivo studies demonstrating the immunosuppressive effects of TLR2 in fungal infections. A study of anti-candidal properties of macrophages has demonstrated that macrophages defective in TLR2 exhibit increased ability to contain *C. albicans* infection [39]. In line with this observation, it has been demonstrated that TLR2−/− mice are more resistant to disseminated candi-
asis, and that this is accompanied by a Th1-bias in these mice [16,18]. C. albicans induces immunosuppression through TLR2-mediated IL-10 release, and this leads to generation of CD4+ CD25+ T-regulatory cells with immunosuppressive potential [16]. Similar data have been reported for schistosomal lyso-phosphatidylserine-induced TLR2 stimulation leading to generation of IL-10-producing T-regulatory cells [40]. The decreased survival after Candida infection in TLR2-/- mice in another study was probably caused by a different experimental design [17]. As with Candida, tolerance induction by Borrelia burgdorferi is conferred through TLR2-mediated release of IL-10, and this has been proposed as a mechanism by which to explain the immunosuppression of chronic Lyme borreliosis, with persistence of the microorganisms in immuno-competent hosts [41]. These effects of TLR2 are reminiscent of those of other pathogen-recognition receptors, such as DC-SIGN or mannose receptors, which also mediate microbial evasion through their interaction with mannose-capped lipoarabinomannan from mycobacteria and induction of a Th2 bias [42,43].

In addition to the induction of anti-inflammatory signals through TLRs, certain fungi have developed strategies to either block or avoid their recognition by TLRs and subsequent activation of the innate defence. Thus, A. fumigatus evades immune recognition by germination into hyphae, with subsequent loss of TLR4-recognition, while the TLR2-mediated IL-10 pathways remain intact, thus shifting the balance towards a permissive Th2-type profile [22]. In recent experiments, it was possible to document a similar evasion of TLR4 recognition by Candida hyphae, which stimulate mainly anti-inflammatory cytokines through TLR2 [44], but which are unable to be recognised by TLR4 and thereby stimulate IL-12 or IFNγ synthesis [44,45].

TLR4 is not the only PRR targeted during fungal germination. In a recent elegant study, Gantrner et al. [46] demonstrated that dectin-1 recognises the β-glucans at the level of budding scars in the yeast, but that it cannot recognise the β-glucans in the hyphae, where they are shielded by a layer of mannans [46]. In this way, two major recognition systems (TLR4 and dectin-1) are unable to recognise candidal hyphae, shifting the balance towards an anti-inflammatory response (Fig. 2).

All these data suggest that fungal pathogens use specific signals induced by PRRs to either down-modulate the microbicidal functions of leukocytes or to escape immune recognition.

CONCLUSIONS

The spectacular discoveries of the last few years in the field of PRRs have demonstrated convincingly that innate immune activation by PRRs is a crucial step in antifungal host defence mechanisms. PRRs recognise PAMPs of fungi, mediate production of cytokines, activate the fungicidal mechanisms of leukocytes, and induce maturation and activation of dendritic cells, thereby providing a bridge between innate and acquired immunity. However, it appears that pathogenic fungi have evolved ways of exploiting part of this recognition system to escape the antifungal host defence mechanisms, either by inducing TLR2-mediated immunosuppression or simply by evading recognition by these classes of receptors.

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