Available online at www.sciencedirect.com
ScienceDirect
Current Opinion in Microbiology

Host–microbe interactions: innate pattern recognition of fungal pathogens

Frank L van de Veerdonk[1,2], Bart Jan Kullberg[1,2], Jos WM van der Meer[1,2], Neil AR Gow[3] and Mihai G Netea[1,2]

The recognition of fungi is mediated by germline pattern recognition receptors (PRRs) such as Toll-like receptors and lectin receptors that interact with conserved structures of the microorganisms, the pathogen-associated molecular patterns (PAMPs). Subsequently, PRRs activate intracellular signals that collaborate for the efficient activation of the host defense. The specificity of these responses is achieved through the activation of a particular mosaic of PRRs, that is determined by the available fungal PAMPs and the innate immune cells involved. This will determine a divergence of the final type of reaction, and in this way the innate host defense has the capability to deliver tailored responses to each pathogen.

Addresses
[1] Department of Medicine, Radboud University Nijmegen Medical Center, Nijmegen, The Netherlands
[2] Nijmegen University Center for Infectious Diseases, Nijmegen, The Netherlands
[3] School of Medical Sciences, Institute of Medical Sciences, University of Aberdeen, Foresterhill, Aberdeen AB25 2ZD, UK

Corresponding author: Netea, Mihai G (m.netea@aig.umcn.nl)

Current Opinion in Microbiology 2008, **11**:305–312

This review comes from a themed issue on
Host-microbe interactions: fungi
Edited by David Underhill

Available online 17th July 2008

1369-5274/$ – see front matter

DOI 10.1016/j.mib.2008.06.002

The essence of the innate defense mechanism is the ability to recognize and eliminate microbial pathogens. This is mediated by a limited arsenal of pattern recognition receptors (PRRs) that are able to recognize conserved structures of microorganisms called pathogen-associated molecular patterns (PAMPs). Several classes of PRRs have been described, among which Toll-like receptors (TLRs), C-type lectin receptors (CLRs), NOD-like receptors, and RigI-helicases. It is especially the first two classes of these receptors that have been suggested to play an important role in antifungal immunity.

Fungal PAMPs

Most components of the fungal cell wall are not found in mammals, and therefore, represent an ideal target for recognition as nonself. The fungal cell wall can be described as a dynamic, highly organized organelle that determines both the shape of the fungus and its viability. In general, the core structure of the fungal cell wall, as exemplified by the structure of *Candida albicans* cell wall, is composed of a skeleton of polysaccharide fibrils composed of β-(1,3)-glucan that is covalently linked to β-(1,6)-glucan and chitin (a β-(1,4)-linked polymer of *N*-acetyl glucosamine), and is designed to function as a scaffold for the external protein layer. This outer layer consists of proteins that are mainly glycosylated through N-linked [1] or O-linked mannosylation [2] (also called mannans). Although this basic model of the fungal cell wall is shared by many fungi, at the molecular level these structures differ between fungal species. In *Aspergillus* species an important component of the skeleton of the cell wall is galactomannan, while the outermost cell wall layer is composed of rodlet fascicles of hydrophobic proteins (hydrophobins) that contribute to the shielding properties of the cell wall [3]. In *Cryptococcus*, a thick capsule of mannoproteins, galactoxylomannan and glucoronoxylomannan plays a crucial role for inhibiting recognition and activation of host defense mechanisms [4]. This diversity will result in different qualities of PRR–ligand interactions and the activation of different sets of PRRs, leading to specific host responses.

The role of PRRs in antifungal host defense

When the host encounters live pathogenic fungi, the initial response by the innate immune system will be determined by the recognition of fungal cell wall components. Neutrophils, monocytes, and macrophages represent the first line of defense against fungal pathogens. Later on, recognition of fungal structures by dendritic cells leads to the activation of specific immunity, especially T-cell-mediated. These various cell populations differ in their expression of TLRs and CLRs on the cell membrane, and are therefore capable of initiating different responses.

TLRs

The first suggestion for a role for TLRs in antifungal host defense was made by Lemaitre *et al.*, who observed that *Drosophila* flies deficient in the Toll receptor rapidly succumbed to *Aspergillus fumigatus* infection, because of defective synthesis of the drosomycin defensin [5]. Ligand recognition by the functionally equivalent TLRs induces the activation of kinase cascades in mammalian cells, and the nuclear translocation of transcription factors such as NF-kB, NF-AT, and IRF3, that induce gene

expression and production of various chemokines and cytokines [6]. Moreover, recently a human homolog of drosomycin called drosomycin-like defensin has been described, that has activity against a variety of filamentous fungi, and is expressed mainly in the skin [7•]. Shortly after the discovery of TLRs, TLR2 and TLR6 were shown to be involved in the recognition of the fungal structure zymosan derived from *Saccharomyces cerevisiae* [8]. Moreover, the adaptor molecule MyD88, that is shared by most TLRs, has proven to be crucial for antifungal defense by several *in vivo* studies [9–11], strongly suggesting that TLRs play a crucial role in host defense against fungi.

TLR4

TLR4 is one of the best studied PRRs, because of its role as the main receptor of bacterial lipopolysaccharides. In a murine experimental infection model of disseminated *C. albicans* infection, it has been shown that the absence of TLR4-mediated signaling resulted in increased susceptibility to the infection, decreased chemokine production, and impaired neutrophil recruitment [12]. The effects on survival were challenged by a later study [13]. Wang *et al.* suggested for the first time a role for TLR4 in the recognition of *A. fumigatus* [14], and subsequent studies have shown that TLR4 is involved in signaling and cytokine production in response to *Aspergillus* [9,15]. These data were supported by a study showing that TLR4$^{-/-}$ mice died significantly sooner when they were infected with *A. fumigatus* conidia [9]. TLR4 is also involved in the susceptibility to *Pneumocystis* pneumonia, with mice deficient in TLR4 having a defective cytokine production by alveolar macrophages leading to increased susceptibility to *Pneumocystis* infection [16]. By contrast, the *C. neoformans* component glucuronoxylomannan binds to TLR4 and leads to translocation of NF-kB, but not to induction of cytokine production [17], and these findings were supported by the fact that TLR4 does not play a major role in cryptococcal host defense [18].

Only limited knowledge exists regarding the nature of the fungal PAMPs that are recognized by TLR4. In addition to the work described above reporting the recognition of glucuronoxylomannan by TLR4, another study reported that TLR4 recognizes mannans from *S. cerevisiae* and *C. albicans* [19]. A subsequent study found that short linear O-bound mannans of *C. albicans* are recognized by TLR4 and induce proinflammatory cytokines such as TNF [20•]. Overall, TLR4 appears to participate in antifungal host defense by recognizing mannan structures and mediating proinflammatory responses.

TLR2

One of the first studies that investigated TLR2 in fungal host defense reported that blocking of TLR2 by specific antibodies resulted in decreased production of TNF and IL-1β after stimulation of monocytes by *C. albicans* [12]. An additional study showed that TLR2$^{-/-}$ mice have a decreased production of TNF and MIP-2, and reduced neutrophil recruitment after a challenge with *Candida* [21]. However, two other studies found that TLR2$^{-/-}$ mice showed an increased resistance to disseminated candidiasis that was accompanied by decreased production of IL-10, and increased IL-12 and INFγ production [9,22]. In line with this, TLR2-deficient macrophages have shown to have an increased ability to contain *C. albicans* [23]. This immunomodulatory effects induced by TLR2 were found to be mediated through the generation of T-regulatory cells with immunosuppressive potential [22,24••]. Evidence for an anti-inflammatory role for TLR2 in antifungal host defense is further supported by a recent study reporting that zymosan can tolerize DCs through a TLR2-mediated and dectin-1-mediated pathway involving MAPK/ERK [25••]. A limited role for TLR1 and especially TLR6, two receptors known for forming heterodimers with TLR2, has been recently reported in case of *C. albicans* recognition [26].

In addition to recognition of *C. albicans*, TLR2 has been suggested to be involved in the recognition of *A. fumigatus* [15]. However, TLR2$^{-/-}$ mice do not have an increased susceptibility to aspergillosis [9], and Wang *et al.* reported no role for TLR2 in the recognition of *Aspergillus* hyphae [14]. The role of TLR2 in the recognition of *C. neoformans* is still not clear, because of differences in the results by the various investigators: one study reported that TLR2 can bind cryptococcal glucuronoxylomannan, but this does not lead to translocation of NF-kB [17]; an additional study found that TLR2 can mediate cytokine production in response to *C. neoformans* [10]; by contrast, Yauch *et al.* found that TLR2 was not involved in cryptococcal-induced cytokine production [11].

In conclusion, TLR2 seems to be involved in the recognition of fungal pathogens, though disagreement persists regarding the precise components recognized, and the amplitude of the effects. Overall, TLR2 ligands seem to induce weaker proinflammatory effects than TLR4 ligands, and TLR2 has also been shown to have immunosuppressive effects, particularly in *C. albicans* host defense, through promoting environments that favor Th2-type or T-reg-type responses [22,24••].

TLR9

Unmethylated CpG sequences are the natural ligands for TLR9, and several reports have now suggested that TLR9 can recognize fungal DNA. Blocking of TLR9 in human monocytes and TLR9-deficient mouse macrophages stimulated with *C. albicans* leads to a reduced production of cytokines, mainly IL-10 [27]. Another study showed that TLR9 detects *A. fumigatus* DNA, resulting in the secretion of proinflammatory cytokines [28]. The same observation has been made for *C. neoformans*

DNA, which was able to trigger IL-12p40 and expression of CD40 upon stimulation in murine DCs [29]. This study also found that cryptococcal DNA activated NF-kB in TLR9-transfected HEK239 cells. However, Bellocchio *et al*. have reported that TLR9$^{-/-}$ mice produced less IL-12 and more IL-4 and IL-10, but this had little effects on the overall mortality of the animals [9]. In conclusion, most of the data available at this time suggest a role for TLR9 for the recognition of fungal DNA, but the magnitude of this effect for the overall antifungal defense is likely to be overshadowed by redundant signals induced by other PRRs.

CLRs

CLRs comprise a large family of receptors, including dectin-1, the macrophage mannose receptor (MR), the dendritic cell-specific ICAM3-grabbing nonintegrin (DC-SIGN), dectin-2, and the circulating mannose-binding lectin (MBL). These receptors share one or more carbohydrate recognition domains that were originally found in the mannose-binding lectin and are evolutionary conserved. Importantly, over the recent years these receptors have been shown to be involved in fungal recognition and the modulation of the innate immune response.

Dectin-1

Dectin-1 recognizes β-(1,3)-glucans through which mediates ligand uptake and phagocytosis, and is able to trigger production of both proinflammatory and anti-inflammatory cytokines [30]. Dectin-1 signals through the kinase Syk and the adaptor CARD9, and this pathway has been shown to induce IL-2 and IL-10 in DCs [31••]. It has also been demonstrated that infection with *C. albicans* induces CARD9-dependent Th-17 cells [31••], and cytokine production induced by *C. albicans* by both human peripheral blood mononuclear cells and murine macrophages is dependent on dectin-1 [32]. Although dectin-1 signaling alone is sufficient to induce responses upon fungal recognition, several studies have emphasized that it is also able to cooperate with TLRs leading to synergistic proinflammatory responses. Two independent studies have shown that dectin-1 in collaboration with TLR2 triggers proinflammatory responses upon stimulation with *C. albicans* and zymosan [33,34], and recently dectin-1 has been found to amplify TLR4-dependent pathways in a Syk-dependent manner [35]. Furthermore, dectin-1 and TLR2 collaborate for the phagocytosis of *Aspergillus* conidia [36], and *A. fumigatus* can activate the transcription factor AP-1 through a dectin-1/Syk-dependent pathway [37].

The first *in vivo* evidence that dectin-1 plays an important role in innate fungal host defense was reported by a study showing that blocking dectin-1 leads to increased *A. fumigatus* fungal burden in the lung [38]. Dectin-1$^{-/-}$ mice are more susceptible to infection with *C. albicans*, resulting in lower survival and increased fungal burdens [39•]. However, another study using a different strain of dectin-1-knockout mice could not confirm this for *C. albicans* infection, but found an increased susceptibility to *Pneumocystis* infection [40•]. No role for dectin-1 for the host defense to *C. neoformans* has been observed [41]. The adaptor molecule CARD9, involved in the dectin-1-signaling pathway, has also been shown to have a crucial role in the survival during disseminated candidiasis [42•]. These data suggest an important role for dectin-1 in antifungal immunity, either directly or through collaborative signaling with TLR2 and/or TLR4.

Macrophage mannose receptor (MR)

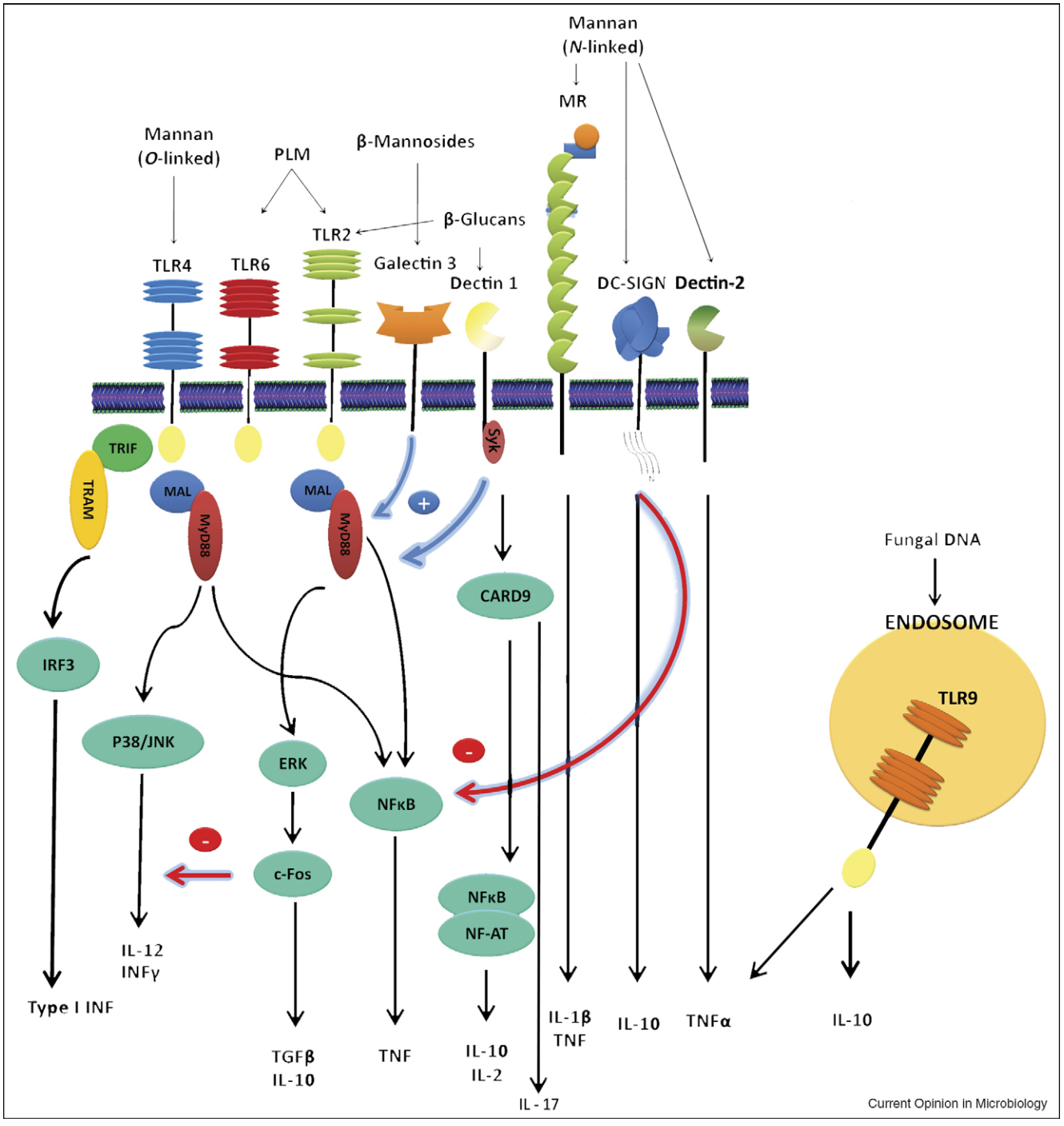
MR has various domains that can recognize oligosaccharides terminating in GlcNAc (chitin is a polymer of GlcNAc), fucose, and mannose. The MR has been implicated in the recognition of several fungi, including *C. neoformans*, *C. albicans*, and *Pneumocystis*. Recently, the role of the MR in *C. albicans* has been investigated using mutant *Candida* strains defective in O-linked and N-linked mannans [20•]. This study showed that the MR recognizes branched N-bound mannans from *C. albicans* and this extended the previous observation that the MR preferentially recognizes branched α-linked oligomannosides [43]. In response to *Pneumocystis* and *C. neoformans*, the MR activates NF-kB and leads to proinflammatory cytokine production [44,45]. However, in the case of *Pneumocystis* the mannose receptor is also capable of inhibiting TNF production, illustrating that the MR can act as a double-edged sword [44].

In vivo data in mice defective for the MR are limited. Although one study suggested only a minor role for MR for the host defense against *Candida* infections [46], this study employed an intraperitoneal model of infection with relatively little relevance to the clinical situation. Another *in vivo* study on *Pneumocystis* infection in MR$^{-/-}$ mice also showed no difference in survival and only small defects in fungal resistance [47].

Other CLRs

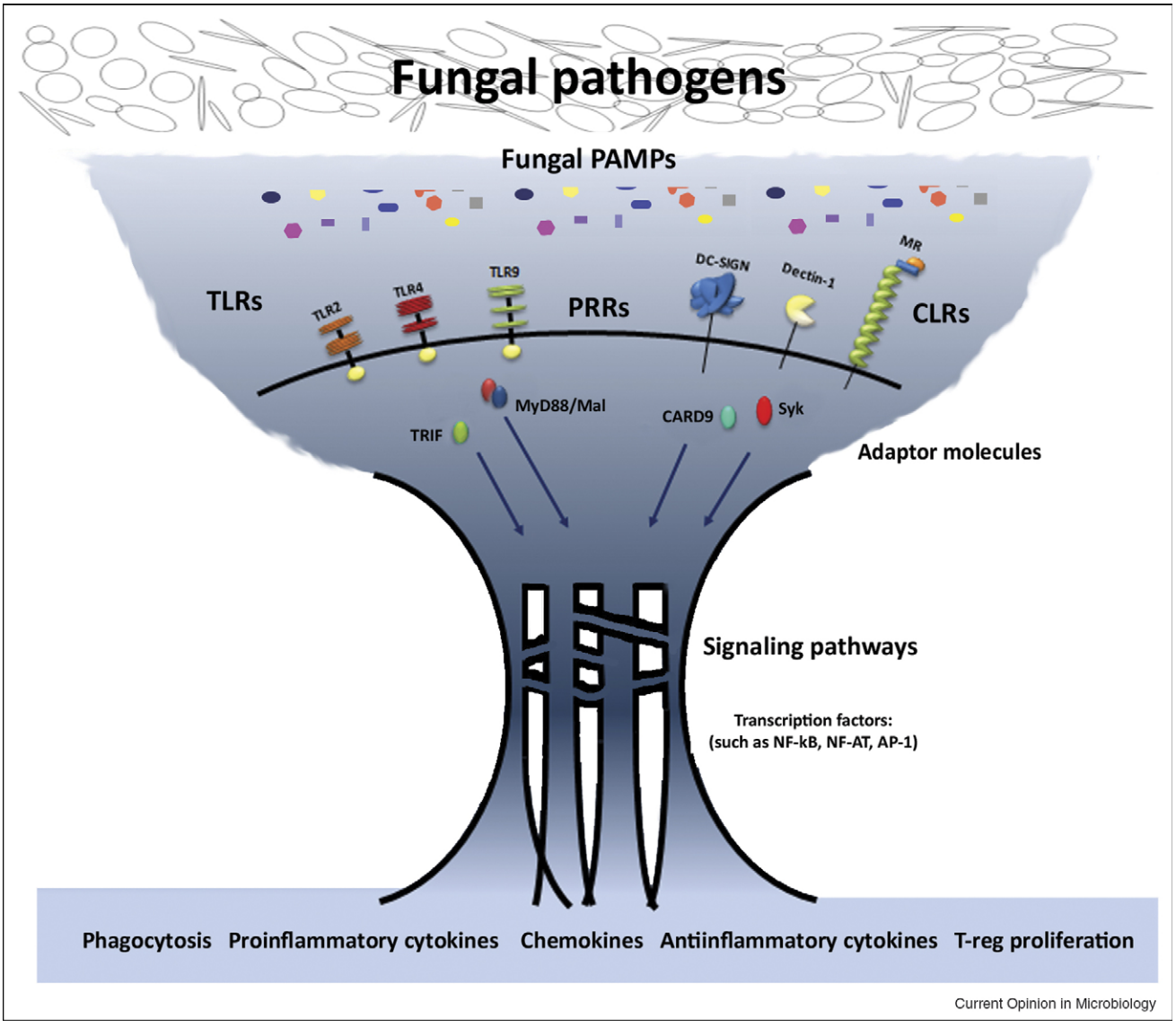
DC-SIGN is primarily expressed on mature DCs and recognizes high-mannose structures in a calcium-dependent way. Recognition has been reported for the pathogenic fungi *C. albicans* [48] and *A. fumigatus* [49] and it mediates uptake and phagocytosis of fungal particles [48]. A recent study suggests an immunosuppressive effect through stimulation of IL-10 production. Dectin-2 is also a member of the CLR family and is mainly present on myeloid cells and maturing inflammatory monocytes, which recognizes high-mannose structures [50] and interacts with the FcγR to induce TNF in response to *C. albicans* hyphae [51]. Dectin-2 can also recognize *Trichophyton rubrum* and *Microsporum audouinii* with preference to their hyphal components. Therefore, dectin-2 mainly seems to play a role in hyphal recognition, and is the first receptor described to produce proinflammatory cytokines

Figure 1



The major pattern recognition pathways of fungal pathogens. Activation of host response by fungal pathogens at the level of the cell membrane is mediated by Toll-like receptors (TLRs) and C-type lectin receptors (CLRs). TLR4 mainly induces proinflammatory signals through the MyD88-Mal-mediated NF-kB and MAPK pathways, while stimulating type I interferons through IRF3. TLR2 has weaker proinflammatory effects, but induces strong stimulation of the anti-inflammatory cytokines IL-10 and TGFβ and can lead to immunological tolarization in DCs through an ERK/MAPK-dependent mechanism. On the contrary, proinflammatory responses induced by TLR2 can be amplified by dectin-1 and galectin-3. Dectin-1 can also induce cytokine production independently of TLR2, and can lead to Th-17 responses through the Syk–CARD9 pathway. The MR induces proinflammatory cytokines such as IL-1β and TNF. DC-SIGN can modulate TLR responses and induces production of IL-10 in DCs. Dectin-2 mainly recognizes mannans from hyphae and leads to the production of TNF.

Figure 2



A general model of fungal pattern recognition. The recognition of the many fungal species is mediated by the interaction between conserved fungal PAMPs and a limited number of PRRs from the TLR and CLR families. These signals further converge because of the use of common adaptor molecules, intracellular pathways, and transcription factors. However, the specificity of the host response is maintained by the different mosaic of receptors stimulated by certain fungi, as well as by the complex interactions between the various pathways. This will determine a divergence of the final type of response elicited by each pathogenic microorganism, and in this way the innate host response has the capability of transforming converging pathways into tailored responses.

in response to fungal hyphae [51]. Galectin-3 is a receptor mainly expressed by macrophages, and it has been shown to be crucial for the recognition of the β-mannosides of *C. albicans*, in close collaboration with TLR2 [52]. Mannose-binding lectin (MBL) is a soluble CLR that is secreted by the liver, which can bind to *C. albicans* [53] and *A. fumigatus* [54]. MBL can also bind to acapsular cryptococcal strains at the level of the budding scar [55]. MBL is mainly involved in fungal host defense because of its ability to opsonize fungal yeasts by activating the complement system [56]. However, MBL-deficient mice do not show decreased survival to infection with *C. albicans* [57] or *A. fumigatus* [58], though a recent study has demonstrated that MBL administration in a murine model of invasive pulmonary aspergillosis can be protective [59]. Recently, a new C-type lectin, Mincle, has been shown to participate in the recognition of *C. albicans* by macrophages. This receptor localizes to the phagocytic cup, but was not essential for phagocytosis. However, knockout mice that lacked this receptor were

hypersusceptible to *Candida* infection, and macrophages in which the Mincle receptor was blocked generated significantly reduced levels of TNF when stimulated by *Candida* yeast cells. The nature of the PAMP that binds to this LR is not yet known [60].

Interactions between PRRs

Initial studies already appreciated that fungi are able to recruit different PRRs to activate specific arms of innate host defense [12]. For example, recognition of *C. albicans* by monocytes and macrophages has been shown to be mediated by at least four recognition systems that sense fungal PAMPs of the *C. albicans* cell wall: recognition of N-linked mannans by MR, recognition of O-linked mannans by TLR4, recognition of β-glucans by dectin-1/TLR2, and recognition of β-mannosides by galectin-3/TLR2 complexes [20•]. If the fungal cell wall is able to trigger many different PRRs at the same, it is important to realize that it is a complex interaction between the various pathways that ultimately leads to the host response.

Several interactions between PRRs are well documented. As mentioned earlier, dectin-1 is able to augment the TLR2-mediated MAPK and NF-kB pathways leading to proinflammatory responses [33,34], and to amplify TLR4 responses through a Syk-dependent pathway [35]. Galectin-3, a PRR which recognizes β-(1,2)-mannosides, has recently been shown to associate with TLR2, and this leads to the ability to discriminate between the pathogenic *C. albicans* and the nonpathogenic *S. cerevisiae* [52]. In addition, the TLR2 pathway itself is able to inhibit TLR4-mediated production of IL-12 through stabilization of c-Fos [61]. Another study demonstrated that when TLRs activate NF-kB, *C. albicans* can induce DC-SIGN-dependent signals which subsequently lead to acetylation of the NF-kB subunit p65 [62]. This results in prolonged and increased IL-10 production that shifts the proinflammatory response induced by TLRs to a more anti-inflammatory response [62]. All these observations imply that crosstalk between PRRs is essential to the complexity and flexibility of the innate immune response against fungi (Figure 1).

Convergence and specificity shape the fungal innate immune response

Although we are still at the beginning of elucidating the combinatorial use of innate defense mechanisms that define the initial host response, a general concept of the innate antifungal defense can be proposed. In order to recognize and respond to the many different fungi the organism encounters, the host evolved germline PRRs that can identify conserved fungal cell wall components — the fungal PAMPs. In this way, specific recognition of fungal nonself is reduced to a handful of specific pathways that interact with each other: the various mannan structures are recognized by TLR4, MR, DC-SIGN, dectin-2, and galectin-3, while the β-glucans are detected by dectin-1, TLR2, and CR3 [63••]. These pathways converge into a limited set of shared adaptor molecules and transcription factors (Figure 2). One such example is that of TLRs and CLRs sharing NF-kB during stimulation of proinflammatory cytokines. However, despite converging into certain pathways, the innate immune response still maintains its specificity through the activation of a specific mosaic of PRRs that is determined by the available fungal PAMPs and the innate immune cells involved. In addition, specificity is also preserved by the interactions between the PRR pathways (Figure 1). This response will eventually lead to nuclear translocation of transcription factors that have the competence to activate specific genes. The specificity insured by these mechanisms will determine a divergence of the final type of response. In this way, the innate host response has the capability of transforming converging pathways into tailored responses (Figure 2).

Conclusions

In this review, we have presented a synthesis of the current knowledge on the recognition of fungal pathogens by the innate immune system of the mammalian host. The very active research of the past few years has greatly improved our understanding of how the fungal pathogens are recognized as nonself by the host defense. Our understanding how TLRs and CLRs contribute and collaborate for the recognition of fungi permitted us to propose an integrated model of innate pattern recognition of these important human pathogens. We have also discussed and speculated how the signals induced by these receptors are integrated to bring about efficient activation of the host innate response. This model that is pertinent conceptually to many host–fungal interactions, may permit in the near future the design of new therapeutic strategies to improve the outcome of patients suffering from these life-threatening infections.

Acknowledgements

MGN was supported by a Vidi Grant of the Netherlands Organization for Scientific Research. NARG is supported by the grants from Wellcome Trust and BBSRC.

References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. Cutler JE: ***N*-Glycosylation of yeast, with emphasis on *Candida albicans***. *Med Mycol* 2001, **39S**:75-86.

2. Ernst JF, Prill SK: ***O*-Glycosylation**. *Med Mycol* 2001, **39(Suppl. 1)**:67-74.

3. Bernard M, Latge J-P: ***Aspergillus fumigatus* cell wall: composition and biosynthesis**. *Med Mycol* 2001, **39(Suppl. 1)**:9-17.

4. McFadden DC, Casadevall A: **Capsule and melanin synthesis in *Cryptococcus neoformans***. *Med Mycol* 2001, **39(Suppl. 1)**:19-30.

5. Lemaitre B, Nicolas E, Michaut L, Reichhart J-M, Hoffmann JA: **The dorsoventral regulatory gene cassette Spaetzle/Toll/Cactus controls the potent antifungal response in *Drosophila* adults**. *Cell* 1996, **86**:973-983.

6. Akira S, Uematsu S, Takeuchi O: **Pathogen recognition and innate immunity**. *Cell* 2006, **124**:783-801.

7. • Simon A, Kullberg BJ, Tripet B, Boerman OC, Zeeuwen P, van der Ven-Jongekrijg J, Verweij P, Schalkwijk J, Hodges R, van der Meer JW *et al.*: **Drosomycin-like defensin, a human homologue of *Drosophila melanogaster* drosomycin with antifungal activity**. *Antimicrob Agents Chemother* 2008, **52**:1407-1412.
This study identifies a homolog of *Drosophila* drosomycin.

8. Ozinsky A, Underhill DM, Fontenot JD, Hajjar AM, Smith KD, Wilson CB, Schroeder L, Aderem A: **The repertoire for pattern recognition of pathogens by the innate immune system is defined by cooperation between Toll-like receptors**. *Proc Natl Acad Sci U S A* 2000, **97**:13766-13771.

9. Bellocchio S, Montagnoli C, Bozza S, Gaziano R, Rossi G, Mambula SS, Vecchi A, Mantovani A, Levitz SM, Romani L: **The contribution of Toll-like/IL-1 receptor superfamily to innate and adaptive immunity to fungal pathogens in vivo**. *J Immunol* 2004, **172**:3059-3069.

10. Biondo C, Midiri A, Messina L, Tomasello F, Garufi G, Catania MR, Bombaci M, Beninati C, Teti G, Mancuso G: **MyD88 and TLR2, but not TLR4, are required for host defense against *Cryptococcus neoformans***. *Eur J Immunol* 2005, **35**:870-878.

11. Yauch LE, Mansour MK, Shoham S, Rottman JB, Levitz SM: **Involvement of CD14, Toll-like receptors 2 and 4, and MyD88 in the host response to the fungal pathogen *Cryptococcus neoformans* in vivo**. *Infect Immun* 2004, **72**:5373-5382.

12. Netea MG, Van Der Graaf CA, Vonk AG, Verschueren I, Van Der Meer JW, Kullberg BJ: **The role of toll-like receptor (TLR) 2 and TLR4 in the host defense against disseminated candidiasis**. *J Infect Dis* 2002, **185**:1483-1489.

13. Murciano C, Villamon E, Gozalbo D, Roig P, O'Connor JE, Gil ML: **Toll-like receptor 4 defective mice carrying point or null mutations do not show increased susceptibility to *Candida albicans* in a model of hematogenously disseminated infection**. *Med Mycol* 2006, **44**:149-157.

14. Wang JE, Warris A, Ellingsen EA, Jorgensen PF, Flo TH, Espevik T, Solberg R, Verweij PE, Aasen AO: **Involvement of CD14 and Toll-like receptors in activation of human monocytes by *Aspergillus fumigatus* hyphae**. *Infect Immun* 2001, **69**:2402-2406.

15. Mambula SS, Sau K, Henneke P, Golenbock DT, Levitz SM: **Toll-like receptor (TLR) signaling in response to *Aspergillus fumigatus***. *J Biol Chem* 2002, **277**:39320-39326.

16. Ding K, Shibui A, Wang Y, Takamoto M, Matsuguchi T, Sugane K: **Impaired recognition by Toll-like receptor 4 is responsible for exacerbated murine Pneumocystis pneumonia**. *Microbes Infect* 2005, **7**:195-203.

17. Shoham S, Huang C, Chen J-M, Golenbock DT, Levitz SM: **Toll-like receptor 4 mediates intracellular signaling without TNF release in response to *Cryptococcus neoformans* polysaccharide capsule**. *J Immunol* 2001, **166**:4620-4626.

18. Nakamura K, Miyagi K, Koguchi Y, Kinjo Y, Uezu K, Kinjo T, Akamine M, Fujita J, Kawamura I, Mitsuyama M *et al.*: **Limited contribution of Toll-like receptor 2 and 4 to the host response to a fungal infectious pathogen *Cryptococcus neoformans***. *FEMS Immunol Med Microbiol* 2006, **47**:148-154.

19. Tada H, Nemoto E, Shimauki H, Watanabe T, Mikami T, Matsumoto T, Ohna N, Tamura H, Shibata K, Akashi S *et al.*: ***Saccharomyces cerevisiae*- and *Candida albicans*-derived mannan induced production of tumor necrosis factor alpha by human monocytes in a CD14- and Toll-like receptor 4-dependent manner**. *Microbiol Immunol* 2002, **2002**:503-512.

20. • Netea MG, Gow NA, Munro CA, Bates S, Collins C, Ferwerda G, Hobson RP, Bertram G, Hughes HB, Jansen T *et al.*: **Immune sensing of *Candida albicans* requires cooperative recognition of mannans and glucans by lectin and Toll-like receptors**. *J Clin Invest* 2006, **116**:1642-1650.
This study characterizes the main pathways of *Candida* recognition by human host defense.

21. Villamon E, Gozalbo D, Roig P, O'Connor JE, Fradelizi D, Gil ML: **Toll-like receptor-2 is essential in murine defenses against *Candida albicans* infections**. *Microbes Infect* 2004, **6**:1-7.

22. Netea MG, Sutmuller R, Hermann C, Van der Graaf CAA, Van der Meer JWM, Adema G, Kullberg BJ: **Toll-like receptor 2 inhibits cellular responses against *Candida albicans* through pathways mediated by IL-10 and CD4+CD25+ regulatory T cells**. *J Immunol* 2004, **172**:3712-3718.

23. Blasi E, Mucci A, Neglia R, Pezzini F, Colombari B, Radzioch D, Cossarizza A, Lugli E, Volpini G, Del Giudice G *et al.*: **Biological importance of the two Toll-like receptors, TLR2 and TLR4, in macrophage response to infection with *Candida albicans***. *FEMS Immunol Med Microbiol* 2005, **44**:69-79.

24. •• Sutmuller RP, den Brok MH, Kramer M, Bennink EJ, Toonen LW, Kullberg BJ, Joosten LA, Akira S, Netea MG, Adema GJ: **Toll-like receptor 2 controls expansion and function of regulatory T cells**. *J Clin Invest* 2006, **116**:485-494.
See annotation to Ref. [25••].

25. •• Dillon S, Agrawal S, Banerjee K, Letterio J, Denning TL, Oswald-Richter K, Kasprowicz DJ, Kellar K, Pare J, van Dyke T *et al.*: **Yeast zymosan, a stimulus for TLR2 and dectin-1, induces regulatory antigen-presenting cells and immunological tolerance**. *J Clin Invest* 2006, **116**:916-928.
Along with Ref. [24••], this study demonstrates that TLR2 induces tolerigenic DCs and Treg proliferation.

26. Netea MG, van de Veerdonk F, Verschueren I, van der Meer JW, Kullberg BJ: **Role of TLR1 and TLR6 in the host defense against disseminated candidiasis**. *FEMS Immunol Med Microbiol* 2008, **52**:118-123.

27. van de Veerdonk, FL., et al.: **Redundant role of TLR9 for anti-*Candida* host defense**. *Immunobiology* 2008, doi:10.1016/j.imbio.2008.05.002.

28. Ramirez-Ortiz ZG, Specht CA, Wang JP, Lee CK, Bartholomeu DC, Gazzinelli RT, Levitz SM: **Toll-like receptor 9-dependent immune activation by unmethylated CpG motifs in *Aspergillus fumigatus* DNA**. *Infect Immun* 2008, **76**:2123-2129.

29. Nakamura K, Miyazato A, Xiao G, Hatta M, Inden K, Aoyagi T, Shiratori K, Takeda K, Akira S, Saijo S *et al.*: **Deoxynucleic acids from *Cryptococcus neoformans* activate myeloid dendritic cells via a TLR9-dependent pathway**. *J Immunol* 2008, **180**:4067-4074.

30. Brown GD: **Dectin-1: a signalling non-TLR pattern-recognition receptor**. *Nat Rev Immunol* 2006, **6**:33-43.

31. •• Leibundgut-Landmann S, Gross O, Robinson MJ, Osorio F, Slack EC, Tsoni SV, Schweighoffer E, Tybulewicz V, Brown GD, Ruland J *et al.*: **Syk- and CARD9-dependent coupling of innate immunity to the induction of T helper cells that produce interleukin 17**. *Nat Immunol* 2007, **8**:630-638.
This study identifies the signaling pathway leading to the induction of Th-17 cells.

32. Gow NAR, Netea MG, Munro CA, Ferwerda G, Bates S, Mora-Montes HM, Walker L, Jansen T, Jacobs L, Tsoni V *et al.*: **Immune recognition of *Candida albicans* beta-glucan by dectin-1 induces cytokines and has non-redundant effects on the activation of innate immunity**. *J Infect Dis* 2007, **196**:1565-1571.

33. Gantner BN, Simmons RM, Canavera SJ, Akira S, Underhill DM: **Collaborative induction of inflammatory responses by dectin-1 and Toll-like receptor 2**. *J Exp Med* 2003, **197**:1107-1117.

34. Brown GD, Herre J, Williams DL, Willment JA, Marshall AS, Gordon S: **Dectin-1 mediates the biological effects of beta-glucans**. *J Exp Med* 2003, **197**:1119-1124.

35. Dennehy KM, Ferwerda G, Faro-Trindade I, Pyz E, Willment JA, Taylor PR, Kerrigan A, Tsoni SV, Gordon S, Meyer-Wentrup F *et al.*: **Syk kinase is required for collaborative cytokine production induced through Dectin-1 and Toll-like receptors**. *Eur J Immunol* 2008, **38**:500-506.

36. Luther K, Torosantucci A, Brakhage AA, Heesemann J, Ebel F: **Phagocytosis of *Aspergillus fumigatus* conidia by murine**

macrophages involves recognition by the dectin-1 beta-glucan receptor and Toll-like receptor 2. *Cell Microbiol* 2007, **9**:368-381.

37. Toyotome T, Adachi Y, Watanabe A, Ochiai E, Ohno N, Kamei K: **Activator protein 1 is triggered by *Aspergillus fumigatus* beta-glucans surface-exposed during specific growth stages**. *Microb Pathog* 2008, **44**:141-150.

38. Steele C, Rapaka RR, Metz A, Pop SM, Williams DL, Gordon S, Kolls JK, Brown GD: **The beta-glucan receptor dectin-1 recognizes specific morphologies of *Aspergillus fumigatus***. *PLoS Pathogens* 2005, **4**:323-334.

39. • Taylor PR, Tsoni SV, Willment JA, Dennehy KM, Rosas M, Findon H, Haynes K, Steele C, Botto M, Gordon S *et al.*: **Dectin-1 is required for beta-glucan recognition and control of fungal infection**. *Nat Immunol* 2007, **8**:31-38.
See annotation to Ref. [40•].

40. • Saijo S, Fujikado N, Furuta T, Chung SH, Kotaki H, Seki K, Sudo K, Akira S, Adachi Y, Ohno N *et al.*: **Dectin-1 is required for host defense against *Pneumocystis carinii* but not against *Candida albicans***. *Nat Immunol* 2007, **8**:39-46.
Along with Ref. [39•], this study demonstrates the role of dectin-1 in *in vivo* antifungal defense.

41. Nakamura K, Kinjo T, Saijo S, Miyazato A, Adachi Y, Ohno N, Fujita J, Kaku M, Iwakura Y, Kawakami K: **Dectin-1 is not required for the host defense to *Cryptococcus neoformans***. *Microbiol Immunol* 2007, **51**:1115-1119.

42. • Gross O, Gewies A, Finger K, Schafer M, Sparwasser T, Peschel C, Forster I, Ruland J: **Card9 controls a non-TLR signalling pathway for innate anti-fungal immunity**. *Nature* 2006, **442**:651-656.
This study demonstrates the role of CARD9 in disseminated candidiasis.

43. Kery V, Krepinsky JJ, Warren CD, Capek P, Stahl PD: **Ligand recognition by purified human mannose receptor**. *Arch Biochem Biophys* 1992, **298**:49-55.

44. Zhang J, Zhu J, Imrich A, Cushion M, Kinane TB, Koziel H: **Pneumocystis activates human alveolar macrophage NF-kappaB signaling through mannose receptors**. *Infect Immun* 2004, **72**:3147-3160.

45. Pietrella D, Corbucci C, Perito S, Bistoni G, Vecchiarelli A: **Mannoproteins from *Cryptococcus neoformans* promote dendritic cell maturation and activation**. *Infect Immun* 2005, **73**:820-827.

46. Lee SJ, Zheng NY, Clavijo M, Nussenzweig MC: **Normal host defense during systemic candidiasis in mannose receptor-deficient mice**. *Infect Immun* 2003, **71**:437-445.

47. Swain SD, Lee SJ, Nussenzweig MC, Harmsen AG: **Absence of the macrophage mannose receptor in mice does not increase susceptibility to *Pneumocystis carinii* infection in vivo**. *Infect Immun* 2003, **71**:6213-6221.

48. Cambi A, Gijzen K, de Vries J, Torensma R, Joosten B, Adema GJ, Netea MG, Kullberg BJ, Romani L, Figdor CG: **The C-type lectin DC-SIGN (CD209) is an antigen-uptake receptor for *Candida albicans* on dendritic cells**. *Eur J Immunol* 2003, **33**:532-538.

49. Serrano-Gomez D, Dominguez-Soto A, Ancochea J, Jimenez-Heffernan JA, Leal JA, Corbi AL: **Dendritic cell-specific intercellular adhesion molecule 3-grabbing nonintegrin mediates binding and internalization of *Aspergillus fumigatus* conidia by dendritic cells and macrophages**. *J Immunol* 2004, **173**:5635-5643.

50. McGreal EP, Rosas M, Brown GD, Zamze S, Wong SY, Gordon S, Martinez-Pomares L, Taylor PR: **The carbohydrate-recognition domain of Dectin-2 is a C-type lectin with specificity for high mannose**. *Glycobiology* 2006, **16**:422-430.

51. Sato K, Yang XL, Yudate T, Chung JS, Wu J, Luby-Phelps K, Kimberly RP, Underhill D, Cruz PD Jr, Ariizumi K: **Dectin-2 is a pattern recognition receptor for fungi that couples with the Fc receptor gamma chain to induce innate immune responses**. *J Biol Chem* 2006, **281**:38854-38866.

52. Jouault T, El Abed-El Behi M, Martinez-Esparza M, Breuilh L, Trinel PA, Chamaillard M, Trottein F, Poulain D: **Specific recognition of *Candida albicans* by macrophages requires galectin-3 to discriminate *Saccharomyces cerevisiae* and needs association with TLR2 for signaling**. *J Immunol* 2006, **177**:4679-4687.

53. Kilpatrick DC: **Mannan-binding lectin: clinical significance and applications**. *Biochim Biophys Acta* 2002, **1572**:401-413.

54. Neth O, Jack DL, Dodds AW, Holzel H, Klein NJ, Turner MW: **Mannose-binding lectin binds to a range of clinically relevant microorganisms and promotes complement deposition**. *Infect Immun* 2000, **68**:688-693.

55. Panepinto JC, Komperda KW, Hacham M, Shin S, Liu X, Williamson PR: **Binding of serum mannan binding lectin to a cell integrity-defective *Cryptococcus neoformans* ccr4Delta mutant**. *Infect Immun* 2007, **75**:4769-4779.

56. Brouwer N, Dolman KM, van Houdt M, Sta M, Roos D, Kuijpers TW: **Mannose-binding lectin (MBL) facilitates opsonophagocytosis of yeasts but not of bacteria despite MBL binding**. *J Immunol* 2008, **180**:4124-4132.

57. Lee SJ, Gonzalez-Aseguinolaza G, Nussenzweig MC: **Disseminated candidiasis and hepatic malarial infection in mannose-binding-lectin-A-deficient mice**. *Mol Cell Biol* 2002, **22**:8199-8203.

58. Hogaboam CM, Takahashi K, Ezekowitz RA, Kunkel SL, Schuh JM: **Mannose-binding lectin deficiency alters the development of fungal asthma: effects on airway response, inflammation, and cytokine profile**. *J Leukoc Biol* 2004, **75**:805-814.

59. Kaur S, Gupta VK, Thiel S, Sarma PU, Madan T: **Protective role of mannan-binding lectin in a murine model of invasive pulmonary aspergillosis**. *Clin Exp Immunol* 2007, **148**:382-389.

60. Wells CA, Salvage-Jones JA, Li X, Hitchens K, Butcher S, Murray RZ, Beckhouse AG, Lo YL, Manzanero S, Cobbold C *et al.*: **The macrophage-inducible C-type lectin, mincle is an essential component of the innate immune response to *Candida albicans***. *J Immunol* 2008, **180**:7404-7413.

61. Agrawal S, Agrawal A, Doughty B, Gerwitz A, Blenis J, van Dyke T, Pulendran B: **Different Toll-like receptor agonists instruct dendritic cells to induce distinct Th responses via differential modulation of extracellular signal-regulated kinase-mitogen-activated protein kinase and c-Fos**. *J Immunol* 2003, **171**:4984-4989.

62. Gringhuis SI, den Dunnen J, Litjens M, van Het Hof B, van Kooyk Y, Geijtenbeek TB: **C-type lectin DC-SIGN modulates Toll-like receptor signaling via Raf-1 kinase-dependent acetylation of transcription factor NF-kappaB**. *Immunity* 2007, **26**:605-616.

63. •• Netea MG, Brown GD, Kullberg BJ, Gow NA: **An integrated model of the recognition of *Candida albicans* by the innate immune system**. *Nat Rev Microbiol* 2008, **6**:67-78.
This is the first description of the integrated model of host/pathogen interaction for a fungal pathogen.