

# An integrated model of the recognition of *Candida albicans* by the innate immune system

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**Abstract** | The innate immune response was once considered to be a limited set of responses that aimed to contain an infection by primitive ‘ingest and kill’ mechanisms, giving the host time to mount a specific humoral and cellular immune response. In the mid-1990s, however, the discovery of Toll-like receptors heralded a revolution in our understanding of how microorganisms are recognized by the innate immune system, and how this system is activated. Several major classes of pathogen-recognition receptors have now been described, each with specific abilities to recognize conserved bacterial structures. The challenge ahead is to understand the level of complexity that underlies the response that is triggered by pathogen recognition. In this Review, we use the fungal pathogen *Candida albicans* as a model for the complex interaction that exists between the host pattern-recognition systems and invading microbial pathogens.

## Innate immune system

The suite of host responses to microbial invaders that results in rapid defence without requiring prior stimulation.

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The clinical spectrum of *Candida* spp. infections ranges from benign colonization of the skin and mucosal surfaces to mucocutaneous forms of candidiasis and systemic infections (candidaemia and deep-seated organ candidiasis). Despite the fact that fungal infections are typically self-limiting, the number of life-threatening systemic fungal infections has risen steadily over the past three decades<sup>1</sup>, although this increase might now be reaching a plateau<sup>2</sup>. The increased prevalence of fungi as agents of disseminated infection has been restricted to patients in whom surgical or chemotherapeutic interventions and/or underlying immunological deficiencies have allowed fungi to overwhelm the protective host defence mechanisms<sup>2</sup>. In healthy and immunologically normal individuals, the innate immune system is an efficient sentinel that provides protection from the thousands of fungal species that humans regularly encounter.

*Candida albicans* is a ubiquitous fungal organism that often colonizes the skin and the mucosal surfaces of normal individuals, without causing disease. However, when the normal host defence mechanisms are impaired (for example, in patients who are undergoing chemotherapy for malignancies, receiving immunosuppressants after an organ transplant, or patients with AIDS), *C. albicans* can become a pathogen. Candidaemia has an incidence of between 1.1 and 24 cases per 100,000 individuals, and an associated mortality of more than 30%<sup>3,4</sup>. It seems

unlikely that antifungal agents will have an impact on the grim mortality statistics for systemic fungal infections without the aid of new clinical approaches, such as combining chemotherapy with immunotherapy<sup>5</sup>. However, augmenting the ability of the immune system to eliminate a pathogen requires a sophisticated understanding of the molecular mechanisms that are involved in pathogen recognition and in the host immune response. In this Review we describe the recent progress that has been made in research into the mechanisms that are involved in the recognition of fungal pathogens, and present how this progress has changed our understanding of the pathogenesis of infections in general, and invasive candidiasis in particular.

## Innate immunity and host defence

Until recently, little was known about the ways in which neutrophils and macrophages, the major players in innate immunity, recognized *C. albicans* as a pathogenic microorganism, or how the fungal–leukocyte interaction triggers an inflammatory response. The dogma that had been blindly accepted over the past 50 years was that although effective, innate immunity was non-specific and “rather primitive and dumb” (REF. 6). However, this simplistic model, in which innate immunity performs only simple ‘ingest and destroy’ tasks, could not explain how innate immune cells recognize microbial pathogens

**Box 1 | The recognition of microorganisms by pattern-recognition receptors**

The innate recognition of pathogens is achieved through germ-line-encoded receptors, the pattern-recognition receptors (PRRs), which sense conserved chemical signatures called pathogen-associated molecular patterns (PAMPs). Four major classes of PRRs have been identified.

- Toll-like receptors (TLRs) are cell-membrane-associated (TLR1, TLR2, TLR4, TLR5 and TLR6) or intracellular (TLR3, TLR7, TLR8 and TLR9) receptors. Several TLRs have been implicated in the recognition of fungal components: TLR2 recognizes phospholipomannan, TLR4 recognizes O-linked mannans, TLR6 is involved in the recognition of zymosan and TLR9 detects fungal DNA.
- C-type lectin receptors (CLRs) are mainly membrane-bound receptors that recognize polysaccharide structures from *Candida albicans*: dectin 1 recognizes  $\beta$ -glucans, whereas the macrophage mannose receptor (MR) and DC-SIGN recognize N-linked mannans.
- Nucleotide-binding oligomerization domain (NOD)-like receptors (NLRs) and retinoic-acid-inducible gene I (RIGI) receptors are intracellular receptors for bacterial peptidoglycans and viral nucleic acids. So far, no studies have documented the involvement of NLRs or RIGI receptors in the recognition of fungi.
- The blueprint for the structure of PRRs is represented by the extracellular pathogen-recognition domain (the Leu-rich repeat (LRR) domain in TLRs and the C-type lectin domain (CLD) in CLRs). The intracellular signalling domain (the TLR-interleukin 1 receptor, TIR domain) of TLRs and the immunoreceptor Tyr-based activation-like motif (ITAM) from some CLRs, such as dectin 1, ultimately induce the intracellular signals responsible for the functional activity of the receptor.

The four major classes of PRRs discussed here are restricted to families of mammalian molecules with a PRR function at a cellular level that have a proven signalling pathway that leads to activation of the innate host response. There are other classes of molecules with less well established functions as PRRs. (For example, peptidoglycan-recognition proteins (PGRPs), which recognize peptidoglycans in insect and mammalian cells. Although PGRPs induce defensins in *Drosophila melanogaster*, their function in mammalian cells has not yet been established.) Some circulating proteins that bind bacterial structures (for example, pentraxins and mannose-binding lectin (MBL)), are also considered by some to be PRRs.

as 'non-self', or why different responses are triggered by different classes of microorganisms. Only in the past decade has it become clear that the innate immune system not only specifically recognizes various classes of microorganisms, it also initiates and modulates the subsequent adaptive responses that are delivered by T-cells and B cells through their interactions with antigen-presenting cells (especially dendritic cells (DCs))<sup>7</sup>. The tasks of recognizing an invading pathogen and activating the host response are accomplished by pattern-recognition receptors (PRRs), which recognize conserved microbial chemical signatures called pathogen-associated molecular patterns (PAMPs) (BOX 1).

**The fungal cell wall**

The fungal cell wall is an essential organelle that maintains the viability of fungal cells. The regulation of fungal cell wall biosynthesis and glycosylation has been extensively reviewed elsewhere<sup>8–10</sup>, but a basic outline of the components of the cell wall is necessary to understand how it is recognized by the host immune system. To be strong, yet plastic, fungal cell walls combine skeletal and matrix components in a way that resembles the engineering principles that are involved in constructing elaborate structures, such as reinforced concrete buildings, that are made of mesh and mortar. The cells of the innate immune system recognize elements of both the skeletal and matrix components of the *C. albicans* cell wall.

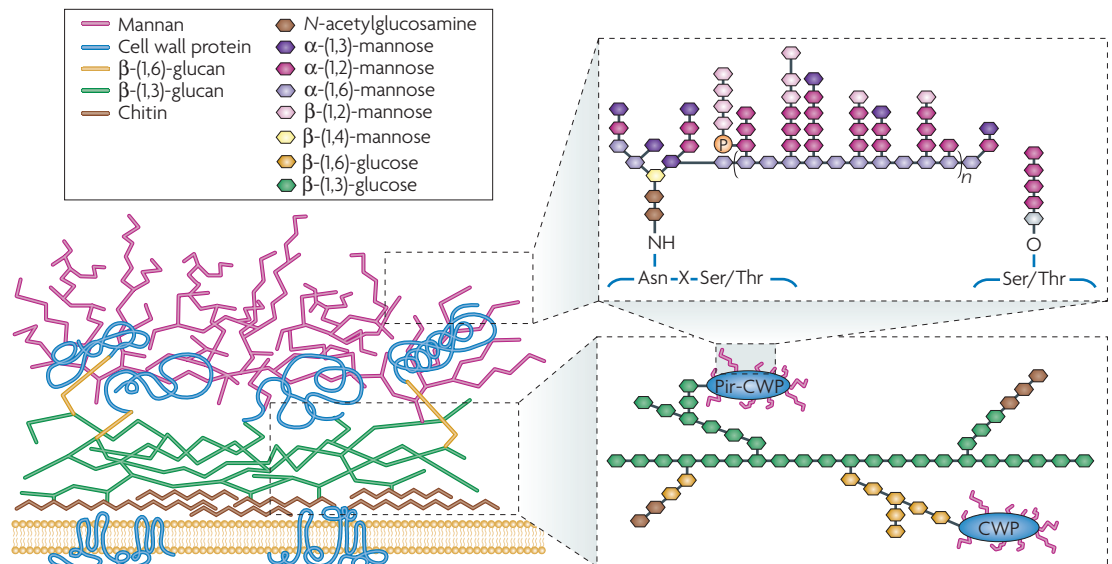
The skeletal component of the cell wall of the majority of fungal pathogens, including *C. albicans*, is based on a core structure of  $\beta$ -(1,3)-glucan covalently linked to  $\beta$ -(1,6)-glucan and chitin (a  $\beta$ -(1,4)-linked polymer of N-acetylglucosamine (GlcNAc)) (FIG. 1). These polymers form hydrogen bonds between adjacent polysaccharide

chains to form a tough three-dimensional network of microfibrils. Most models suggest that the skeletal components of the cell wall are found close to the cell membrane in an inner layer, although some chitin and glucan can be present throughout the thickness of the wall. In budding yeast cells, a scar is left on the mother cell after separation of the bud, and at this site the components of the inner layers of the cell wall, such as chitin and  $\beta$ -(1,3)-glucan, can become exposed at the surface<sup>11</sup>.

In addition to the glucan and chitin skeleton, the *C. albicans* cell wall contains a matrix that mainly comprises glycosylated proteins. In *C. albicans*, the major class of cell wall proteins are glycosylphosphatidylinositol (GPI)-anchor-dependent cell wall proteins (GPI-CWPs), which are attached through a GPI remnant to  $\beta$ -(1,3)-glucan or chitin by a highly branched  $\beta$ -(1,6)-glucan linker. The CWPs are normally highly glycosylated with mannose-containing polysaccharides (sometimes called mannan), and carbohydrates can account for up to 90% of their molecular mass. Many CWPs have a lollipop structure with a globular domain that is presented to the outside of the cell and a Ser/Thr-rich polypeptide stem-like domain that is stabilized in the cell wall by O-linked mannan side chains<sup>8</sup>. These ether-linked O-mannans are relatively short, linear polysaccharides that, in *C. albicans*, consist of one to five mannose (Man) sugars that are almost exclusively  $\alpha$ -(1,2)-linked (FIG. 1). N-mannan consists of a core Man<sub>8</sub>GlcNAc<sub>2</sub> triantennary complex to which a highly branched structure is attached, comprising up to 150 mannose sugars arranged as an  $\alpha$ -(1,6)-linked backbone with side chains of  $\alpha$ -(1,2)-,  $\alpha$ -(1,3)-mannose and phosphomannan<sup>9</sup> (FIG. 1). The mannan structures define many of the serotypes of *Candida* spp.<sup>12</sup> For example, in serotype B strains

**Dendritic cells**

'Professional' antigen-presenting cells that are found in the T-cell areas of lymphoid tissues and as minor cellular components in most tissues. They have a branched or dendritic morphology and are important stimulators of T-cell responses.



**Figure 1 | The structure of the *Candida albicans* cell wall.** The schematic shows the major components of the cell wall and their distributions.  $\beta$ -(1,3)-glucan and chitin (poly- $\beta$ -(1,4)-*N*-acetylglucosamine) are the main structural components, and these are located towards the inside of the cell wall. The outer layer is enriched with cell wall proteins (CWP) that are attached to this skeleton mainly via glycosylphosphatidylinositol remnants to  $\beta$ -(1,6)-glucan or, for mannoproteins with internal repeat domains (Pir-CWP), via alkali-sensitive linkages to  $\beta$ -(1,3)-glucan. The insets show the structure of the glucan and mannan components.

$\beta$ -(1,2)-mannose is found exclusively in the phosphomannan, whereas in serotype A strains  $\beta$ -(1,2)-mannose also occurs in the acid-stable side-chain fraction<sup>9</sup>.

### Fungal recognition

Owing to the localization of mannoproteins and mannans in the outermost part of the cell wall, mannan detection would be expected to be one of the first steps in the recognition of *C. albicans* by the host innate immune system. However, the presence of  $\beta$ -glucans and chitin, especially at the level of the bud scar, is also likely to influence the recognition of *C. albicans* by leukocytes. So how is the recognition of the *C. albicans* cell surface achieved by the immune system?

The main cells of the host innate immune response that recognize invading pathogens are monocytes and neutrophils in the circulation, together with macrophages in infected tissues. Monocytes express high levels of Toll-like receptors (TLRs) on their cell membranes, as well as moderate levels of lectin receptors (LRs). During differentiation into macrophages, they retain expression of TLRs while strongly upregulating their expression of LRs. Important differences in the expression of several receptors have also been reported between resident and inflammatory macrophages, as the expression of PRRs can be modified by cytokines or microbial products. DCs, which are crucial for antigen processing and presentation, also express most of the PRRs that are important for the recognition of fungal pathogens. Neutrophils show moderate expression of TLRs and strongly express phagocytic receptors such as complement receptor 3 (CR3) and  $Fc\gamma$  receptors ( $Fc\gamma$ Rs), but the expression of PRRs on T cells is much more restricted (FIG. 2). The mosaic of PRRs that is expressed by each of these cell

types ultimately determines the type of response that is initiated following recognition of *C. albicans*.

**Mannans and mannoproteins.** Both mannans and mannoproteins from the *C. albicans* cell wall have important immunostimulatory activities, ranging from stimulation of cytokine production<sup>13,14</sup> to induction of DC maturation<sup>15</sup> and T-cell immunity<sup>16,17</sup>. Mannoproteins induce mainly T helper 1 ( $T_H1$ )-type cytokine profiles, which have protective effects against disseminated *C. albicans* infection<sup>18</sup>.

The first receptor on the surface of macrophages to be described as a mannan receptor was the C-type-lectin mannan receptor (MR)<sup>19,20</sup>. The MR recognizes oligosaccharides that terminate in mannose, fucose and GlcNAc<sup>21</sup>, and this binding is mediated by carbohydrate-recognition domains (CRDs) 4 to 8 in the extracellular region of the receptor<sup>22</sup>. *In vitro* studies have shown that the MR preferentially recognizes  $\alpha$ -linked oligomannoses with branched, rather than linear, structures<sup>23</sup>, and this was supported by data that demonstrated that in monocytes and macrophages, the MR recognizes branched *N*-bound mannans from *C. albicans*<sup>24</sup>. By contrast, recognition of the shorter linear structures of *O*-bound mannan is performed by TLR4 (REF. 24), and results in cytokine production<sup>25</sup>. Interestingly, TLR4 stimulation is lost during the germination of yeast into hyphae, which leads to a loss of interferon- $\gamma$  (IFN $\gamma$ ) production capacity<sup>26</sup>. On DCs, however, the binding of *C. albicans* mannans is mediated through the MR and DC-SIGN (DC-SIGN is a receptor that is specifically expressed on the cell membrane of DCs)<sup>27</sup>.

The  $\alpha$ -linked mannose structures on the surface of *C. albicans* are recognized by the MR, TLR4 and

#### Cytokines

Biologically active molecules that are released by cells and that affect the function of other cells.

#### $Fc\gamma$ receptor

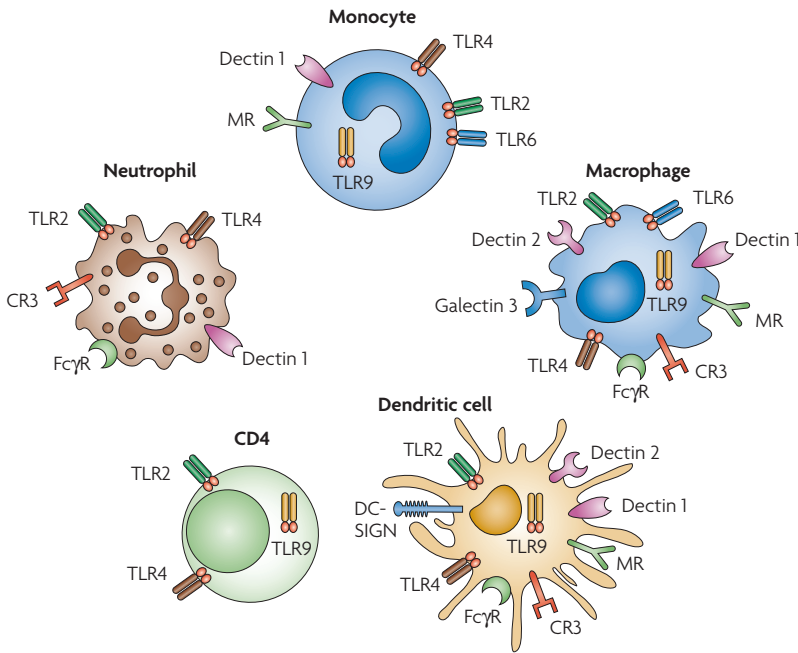
A surface molecule on various cells that binds to the Fc regions of immunoglobulins, thereby initiating effector functions.

#### T helper 1

An immune response that is characterized by a subset of helper T-cells that secrete a particular set of cytokines, including interleukin 2, interferon- $\gamma$  and TNF $\alpha$ , the main function of which is to stimulate phagocytosis-mediated defences against intracellular pathogens.

#### C-type lectin

C-type lectins are largely calcium-dependent animal lectins that are carbohydrate-binding proteins. The binding activity of C-type lectins is based on the structure of the carbohydrate-recognition domain, which is highly conserved in this family.



**Figure 2 | Cell populations and pattern-recognition receptors involved in *Candida albicans* recognition.** The main populations involved in the recognition of *C. albicans* during the innate immune response are the monocytes, neutrophils and macrophages. Dendritic cells are crucial for processing of, and antigen presentation to, T cells, and thus to activation of specific immunity. The differential expression of pattern-recognition receptors by these cell types is shown. CR3, complement receptor 3; FcγR, Fcγ receptor; MR, mannose receptor; TLR, Toll-like receptors.

DC-SIGN. By contrast, β-(1,2)-mannosides, which are present in the acid-stable and acid-labile components of mannoproteins and in phospholipomannan (PLM), are recognized by other mechanisms. On the one hand, PLM reportedly stimulates cytokine production through TLR2 (REF. 28). On the other hand, it has been suggested that recognition of the acid-labile β-(1,2)-mannosides is redundant in human monocytes<sup>24</sup>, although it might play a role in tissue macrophages, particularly in the gut mucosa. In this respect, a recent study has shown that *galectin 3* on the surface of murine macrophages can discriminate between pathogenic *C. albicans* and non-pathogenic *Saccharomyces cerevisiae*, and that an association between *galectin 3* and TLR2 is involved in this process<sup>29</sup>.

Another lectin family member, *dectin 2*, has also been described to function as a receptor for *C. albicans* mannans<sup>30</sup>, although this interaction is less well characterized. Dectin 2 is mainly expressed on myeloid cells and maturing inflammatory monocytes<sup>31</sup>. Owing to its short intracytoplasmic tail, dectin 2 must interact with the FcγR to induce intracellular signals and, interestingly, seems to be mainly involved in the recognition of *C. albicans* hyphae<sup>32</sup>. Dectin 2 is encoded by a different gene cluster to the β-glucan receptor *dectin 1* (see below) and does not recognize β-glucans<sup>32</sup>; rather, dectin 2 has binding specificity for mannose<sup>33</sup>.

**β-glucans.** The *C. albicans* cell wall consists of approximately 60% β-glucan<sup>34</sup>. Although initially thought to be buried beneath the mannoprotein layer, recent evidence

suggests that β-glucans are exposed on the cell surface<sup>35</sup>, although possibly restricted to specific regions, such as bud scars<sup>11</sup>. β-glucans can stimulate leukocytes *in vitro*, which induces cytotoxic and antimicrobial activities as well as the production of pro-inflammatory mediators, cytokines and chemokines<sup>36</sup>. β-glucans are released into the circulation during systemic fungal infections<sup>37</sup>.

The recognition of β-glucans has primarily been ascribed to two receptors, CR3 and, more recently, dectin 1. Although other β-glucan receptors have been described, including lactosylceramide and scavenger receptors, the physiological role of these receptors is still unclear. CR3 is a widely expressed β<sub>2</sub>-integrin that recognizes several endogenous and exogenous ligands, pathogens that have been opsonized by iC3b (the inactivated form of complement component C3b) and carbohydrates, including β-glucans. Carbohydrate recognition is mediated by a lectin domain<sup>38,39</sup>, which is distinct from the normal ligand-binding site (the I domain) of CR3 (REF. 39). The lectin domain mediates recognition of both the yeast and hyphal forms of *C. albicans*<sup>40,41</sup>, as well as several other fungi. Recognition by CR3 does not trigger protective host responses, such as the respiratory burst<sup>42</sup>, and can repress pro-inflammatory signals<sup>43,44</sup>.

Dectin 1 is a myeloid-expressed transmembrane receptor and possesses a single extracellular, non-classical C-type-lectin-like domain that specifically recognizes β-(1,3)-glucans. Dectin 1 can recognize several fungi, including *C. albicans* yeast, although it does not appear to recognize *C. albicans* hyphae<sup>11,45</sup>. The cytoplasmic tail of dectin 1 contains an immunoreceptor tyrosine-based activation-like motif (ITAM), which can mediate various protective responses through spleen tyrosine kinase and caspase recruitment domain protein 9 (*Syk-CARD9*)-dependent pathways, such as the stimulation of interleukin 2 (*IL-2*) and *IL-10* (REF. 46), *IL-6* (REF. 47), and *IL-17* production<sup>48</sup>. Although *Syk*-dependent signalling from dectin 1 is sufficient for these responses, stimulation of the mitogen-activated protein kinase (MAPK) and nuclear factor (NF)-κB pathways, with subsequent production of pro-inflammatory cytokines, such as tumour necrosis factor (TNF), requires collaborative signalling with the TLR2 receptor<sup>49,50</sup>. The precise nature of the TLR2 ligand from *C. albicans* has not yet been completely elucidated, although PLM might be recognized by TLR2 and TLR6 (REF. 28).

It has recently been suggested that phagocytosis of *C. albicans* by neutrophils can be mediated by the minor cell wall component β-(1,6)-glucan<sup>51</sup>. Beads coated with β-(1,6)-glucan are ingested by neutrophils, and the phagocytosis of yeast cells treated with β-(1,6)-glucanase is reduced. This recognition appears to be mediated by CR3 following opsonization by C3d fragments that bind β-(1,6)-glucan.

**Other *C. albicans* components.** In addition to mannoproteins, mannans and β-glucans, other structures of *C. albicans* can also be recognized as fungal PAMPs. Chitin, a less studied polysaccharide component of the *C. albicans* cell wall, is a β-(1,4)-linked homopolymer of GlcNAc that forms antiparallel hydrogen-bonded chains

**Chemokines**  
Small, inducibly secreted proteins that induce the activation and migration of lymphocytes.

**Complement**  
A part of the innate immune system that comprises serum proteins that can protect against infection.



called microfibrils<sup>52</sup>. Recently, it has been demonstrated that chitin induces recruitment of immune cells that mainly release IL-4 and IL-13 (REF. 53). Little is known about the recognition pathways of chitin and its role during *C. albicans* infections, and no recognition receptors for chitin have yet been described.

Bacterial and fungal DNA are poorly methylated, in contrast to mammalian DNA. This difference has been proposed to be instrumental in the recognition of non-self DNA by TLR9 (REF. 54). Although the recognition of fungal DNA has not been formally demonstrated, the involvement of TLR9 in the recognition of *C. albicans* is supported by the observation that cytokine production from CD4<sup>+</sup> T-cells from TLR9<sup>-/-</sup> mice is skewed (higher IL-4, lower IFN $\gamma$ ) compared to cytokine production from CD4<sup>+</sup> T cells from wild-type mice, upon challenge with *C. albicans* yeast<sup>55</sup>. As yet, no studies have investigated the possible role of RNA recognition systems (that is, TLR3, and TLR7 and TLR8) in the host response to *C. albicans* infection.

### Activation of host defence by PRRs

At first glance, ligation of the various host immune receptors by *C. albicans* PAMPs appears to lead to a set of standard, and possibly redundant, pathways that stimulate cytokine production, phagocytosis and fungal killing. However, this early model of a 'standard' PRR response has been refined over the past few years. Various PRRs enable the innate immune system not only to recognize specific PAMPs, but also to specifically modulate the response that follows. In addition, by inducing specific cytokine profiles, PRRs bring a certain degree of specificity to the innate response.

**C. albicans uptake.** Phagocytosis of *C. albicans* is mediated by the concerted action of several opsonic and non-opsonic receptors. Complement binding and activation is mediated by the alternative pathway, and complement activation is mainly important for the chemotaxis and opsonization of *C. albicans*, but not for *C. albicans* lysis, which is prevented by the thick and complex cell wall<sup>56</sup>. Furthermore, although mannose-binding lectin does bind and recognize *C. albicans*, the lectin pathway of complement activation probably plays only a minor role in *C. albicans* uptake<sup>57</sup>.

Several membrane-bound receptors contribute to the phagocytosis of *C. albicans*, among which dectin 1 (REF. 58), the MR<sup>59,60</sup> and DC-SIGN<sup>27</sup> have been directly shown to mediate uptake of fungal particles in transfected cell systems, although the ability of the MR to mediate phagocytosis has recently been questioned<sup>61</sup>. TLRs do not mediate fungal uptake, but they might be involved in directing the subsequent maturation of the phagosome<sup>62</sup> and the presentation of antigens, although this is still controversial<sup>62</sup>.

**C. albicans killing.** Following uptake, killing of *C. albicans* occurs through both oxidative and non-oxidative mechanisms<sup>63</sup>. Although the receptors that are involved in triggering these events are largely unknown, dectin 1 induces the respiratory burst in response to

fungi, an activity that can be enhanced by TLR signaling<sup>50,64</sup>. The respiratory burst is an essential antifungal effector mechanism that results in the production of toxic oxidants<sup>65,66</sup> and in the activation of granule proteases that can kill *C. albicans*<sup>65-67</sup>. Killing of *C. albicans* also occurs extracellularly, through the as-yet-undefined actions of PRRs such as galectin 3 (REF. 68).

**Cytokine induction.** More is known about the receptors that are involved in the induction of cytokine production by *C. albicans*. At least four TLRs (TLR2, TLR4, TLR6 and TLR9) are involved in triggering these responses. Upon recognition of microbial structures, TLRs activate either the NF- $\kappa$ B or MAPK pathway, which lead to the stimulation of pro-inflammatory cytokine production<sup>69</sup>. The balance between the signals that are induced by TLR2 and TLR4 seems to have a crucial role in the regulation of the immune response. TLR4 can strongly stimulate pro-inflammatory cytokines through two pathways: one involves the myeloid differentiation primary response gene 88 (MyD88)-Mal-mediated induction of the NF- $\kappa$ B-dependent release of pro-inflammatory cytokines and chemokines; the other involves the interferon regulatory factor 3 (IRF3)-dependent release of type I interferons<sup>70</sup>, which induce the secondary production of T<sub>H</sub>1-type cytokines such as IFN $\gamma$ <sup>26,55,71</sup>.

By contrast, several studies have demonstrated that although TLR2 ligation can induce pro-inflammatory cytokines, this effect is weaker than that mediated by TLR4 ligation<sup>72</sup>. Moreover, TLR2 ligands fail to induce the release of IL-12 and T<sub>H</sub>1-type IFN $\gamma$ , thus promoting conditions that are favourable for T<sub>H</sub>2- or regulatory T cell (T<sub>Reg</sub>)-type responses<sup>73</sup>. Stabilization of the transcription factor c-Fos, which is a suppressor of IL-12 expression, is an important step in this process<sup>74</sup>. In support of this, a recent *in vitro* study has reported that zymosan induces the development of a tolerogenic DC population through TLR2 and dectin 1 (REF. 75). The induction of this tolerogenic cytokine profile depends on extracellular signal related kinase (ERK)/MAPK phosphorylation, a mechanism that is distinct from the p38/Jun N-terminal kinase (JNK) pathway that is induced by TLR4 stimulation<sup>75,76</sup>. Also, *in vivo* studies have shown that knockout mice that lack TLR2 are more resistant to disseminated candidiasis, and this is accompanied by a T<sub>H</sub>1 bias<sup>55,77</sup>. *C. albicans* induces immunosuppression through TLR2-mediated IL-10 release, and this leads to the generation of CD4<sup>+</sup>CD25<sup>+</sup> T<sub>Reg</sub> cells with immunosuppressive potential<sup>77,78</sup>. Similar data have been reported in models of *Schistosoma mansoni* and *Borrelia burgdorferi* infection<sup>79,80</sup>.

The role of TLR6 and TLR9 in the induction of cytokines is less prominent. Although TLR2-TLR6 heterodimers are involved in the recognition of zymosan<sup>81</sup>, cytokine production is only moderately reduced in TLR6<sup>-/-</sup> macrophages that are stimulated with *C. albicans*, and this does not result in increased susceptibility to disseminated candidiasis<sup>82</sup>. Similar to TLR2, the absence of TLR9 also results in a slight shift of the cytokine production that is induced by *C. albicans* towards a more anti-inflammatory profile<sup>55</sup>.

#### Type I interferons

Interferons that are rapidly induced by virus replication, as well as by some bacterial and fungal infections.

#### T<sub>H</sub>2

A type of activated T helper cell that participates in phagocytosis-independent responses and that downregulates pro-inflammatory responses that are induced by T<sub>H</sub>1 cells. T<sub>H</sub>2 cells secrete interleukin 4 (IL-4), IL-5, IL-6 and IL-10.

#### Regulatory T-cell

A small population of CD4<sup>+</sup> T-cells that expresses the transcription factor forkhead box P3 and that has suppressor activity towards other T-cells either by cell-cell contact or cytokine release.

#### Zymosan

Particulate preparation of *S. cerevisiae* cell walls that is rich in  $\beta$ -glucans and mannan and that can be used to activate the innate immune system.

Of the non-TLR receptors, dectin 1, dectin 2 and the MR have been implicated in initiating responses to *C. albicans*. Not much is known about dectin 2, but this receptor does preferentially bind *C. albicans* hyphae and induces the production of TNF $\alpha$  and IL-1 receptor antagonist (IL-1Ra)<sup>32</sup>. Dectin 1 induces the production of numerous cytokines and chemokines in response to fungi, including TNF $\alpha$ , macrophage inflammatory protein 2 (MIP2), macrophage inflammatory protein 1 $\alpha$  (MIP1 $\alpha$ ), granulocyte-macrophage colony-stimulating factor (GM-CSF), granulocyte colony-stimulating factor (G-CSF), IL-10, IL-2, IL-1 $\alpha$ , IL-1 $\beta$ , IL-23 and IL-6 (REFS 46,50,83–86). Whereas the production of IL-10, IL-6 and IL-2 can be mediated by dectin 1 directly<sup>87</sup>, the induction of pro-inflammatory cytokines and chemokines requires collaborative signalling with TLR2 (REFS 50,83), although evidence suggests that dectin 1 is sufficient for these responses in certain cell types, such as alveolar macrophages<sup>85</sup>. By contrast, the interaction of dectin 1 with the tetraspanin CD37 seems to inhibit the function of dectin 1 and stimulation of IL-6 (REF. 88).

The intracellular pathways that are activated by dectin 1 for the induction of cytokines involve the recruitment of Syk<sup>46</sup> and downstream signalling via CARD9 (REF. 89). The dectin 1–Syk–CARD9 pathway induces DC maturation and directs T<sub>H</sub>17 responses that are independent of the interaction with TLRs<sup>48</sup>, and this effect has been particularly linked with hyphal stimulation of DCs<sup>90</sup>. The role of IL-17 in the host response to disseminated candidiasis has been suggested to be protective, by inducing neutrophil recruitment<sup>91</sup>, whereas it exerts a predominantly inflammatory pathological effect in gastric *C. albicans* infections<sup>92</sup>. In addition to its mediation of the dectin 1–Syk pathway, CARD9 is also a central adaptor molecule that mediates the pro-inflammatory signals that are induced by other classes of receptors, such as the nucleotide-binding oligomerization domain (NOD)-like receptors<sup>93</sup>, ITAM-associated receptors and possibly the TLRs<sup>94</sup>. Recently it has also been suggested that TRIF-dependent pathways modulate the balance between deleterious T<sub>H</sub>17 responses and protective T<sub>Reg</sub> cells in mice with gastric candidiasis<sup>95</sup>.

The receptors and intracellular pathways that are involved in the recognition of *C. albicans* by leukocytes are depicted in FIG. 3. Although all of these pathways have been demonstrated to be involved in the recognition of fungal components, it has not been verified that identical mechanisms are responsible for the recognition of intact, live *C. albicans* cells. For example, zymosan has been used extensively for immune stimulation, but the relationship between the response to zymosan and the response to native fungal cells has not yet been clarified. The specificity of the antifungal response is evident in differences between the kinetics of the various immunological effects and in the magnitude of each of these responses.

Epithelial and endothelial surfaces also have PRRs that recognize surface PAMPs of invading and colonizing microorganisms such as *C. albicans*. Studies of the role of epithelial cell immunity have been significantly enhanced by the availability of model epithelial and endothelial tissues in which a cell line of keratinocytes is grown on the

surface of a polycarbonate support membrane. Immune cells such as polymorphonuclear leukocytes (PMNs) can be added to such *ex vivo* reconstituted human epithelial (RHE) tissue cultures along with an inoculum of fungal cells. The expression of TLRs in RHE models is similar to the TLR expression profile *in vivo*<sup>96</sup>. Using such approaches, PMNs have been shown to be active in the innate immune response at these primary barriers to systemic invasion<sup>97</sup>, and differences have been noted between the local immunity of the oral and vaginal mucosae<sup>97,98</sup>. For example, PMNs enhance an inflammatory T<sub>H</sub>1 response in the oral epithelia, which results in the induction of pro-inflammatory cytokines, such as IFN $\gamma$  and TNF $\alpha$ , effectively mimicking the situation that is observed *in vivo*<sup>97</sup>. Vaginal epithelial cells express TLR2 and TLR4 *in vivo*, and the presence of heat-inactivated *C. albicans* cells and zymosan induces these cells to secrete inflammatory cytokines, chemokines and to produce  $\beta$ -defensin 2 — an antimicrobial compound that also induces PMN chemotaxis<sup>99</sup>. Work to characterize fungal PAMPs and PRRs that induce local innate responses is ongoing. For example, it has recently been shown that human epithelial TLR4 is directly involved in the PMN-dependent protection of oral mucosa<sup>96</sup>.

#### **In vivo models in TLR- and LR-deficient mice**

*In vitro* stimulation models that use either cell lines or primary cells have suggested specific roles for the various PRRs in the recognition of *C. albicans*, as detailed above. However, whether these pathways are indeed important for the antifungal host response *in vivo* can only be demonstrated in experimental models of infections in genetically modified mice that lack particular receptors, or in patients with specific immune defects.

Since the first observation that TLRs recognize *C. albicans* and activate the innate immune response<sup>74</sup>, additional studies have confirmed the important role that TLRs have in the recognition of *C. albicans* and the anti-candidal host response. A global role for TLRs in defence against disseminated candidiasis was demonstrated by the increased susceptibility of MyD88<sup>-/-</sup> mice to *C. albicans* infection<sup>55,100,101</sup>. A subsequent study reported increased susceptibility of TLR2<sup>-/-</sup> mice to disseminated candidiasis, and this effect was attributed to decreased production of TNF and chemokines<sup>102</sup>. However, the increased susceptibility of TLR2<sup>-/-</sup> mice to infection with *C. albicans* was not confirmed in two other studies, which found reduced mortality and a decreased fungal burden in TLR2<sup>-/-</sup> mice, accompanied by decreased production of IL-10 and increased production of IL-12 and IFN $\gamma$ <sup>55,77</sup>. An additional *in vitro* study also confirmed the increased ability of macrophages from TLR2<sup>-/-</sup> mice to contain *C. albicans*<sup>103</sup>. These latter studies<sup>55,77,103</sup> suggest that TLR2, by inducing mainly an anti-inflammatory response, inhibits the innate immune response to *C. albicans* infections in certain circumstances.

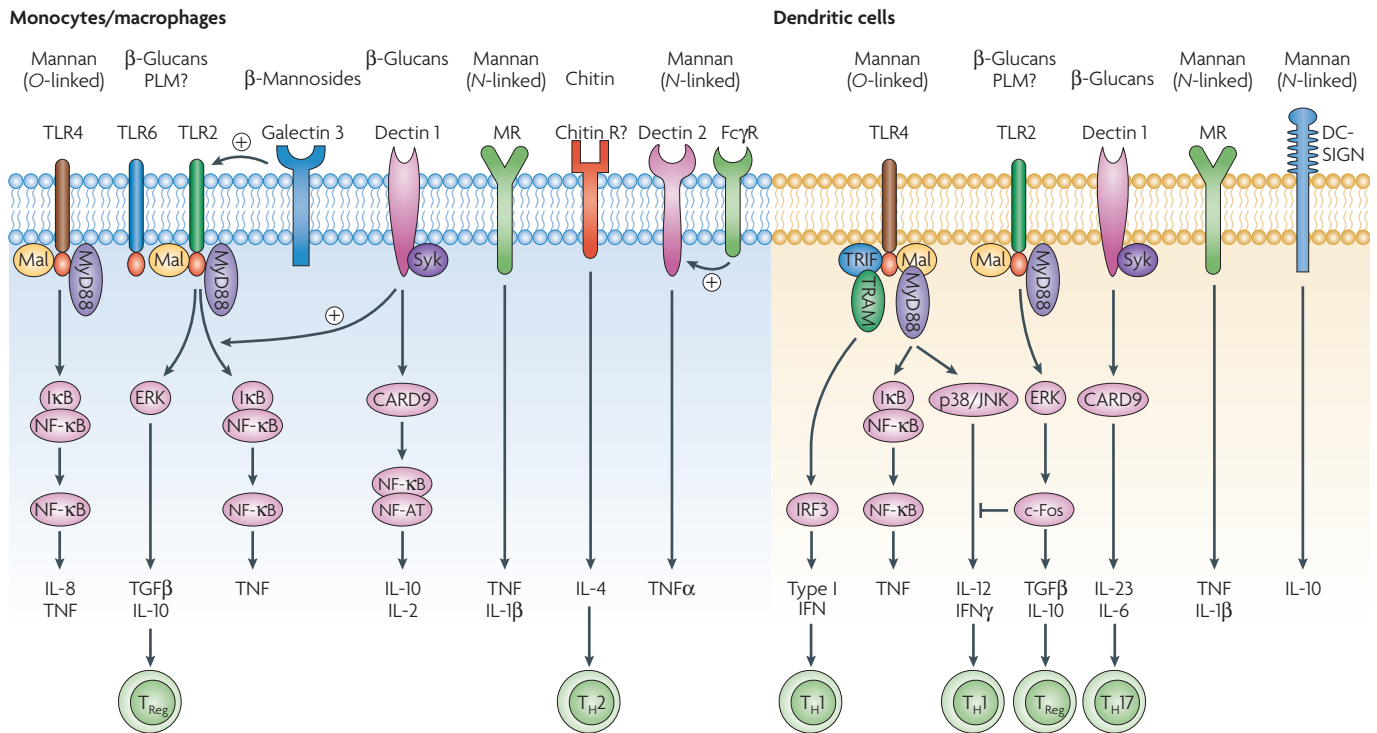
It has also been suggested that TLR4 is involved in the recognition of *C. albicans*<sup>24,25,71</sup>, and TLR4-defective C3H/HeJ mice have been reported to be more susceptible to disseminated candidiasis<sup>71</sup>. However, other studies

#### **Tetraspanin**

A family of transmembrane proteins that have four transmembrane domains and two extracellular domains of different sizes. The function of tetraspanins is unclear, but they seem to interact with many other transmembrane proteins and form large multimeric protein networks.

#### **T<sub>H</sub>17 response**

The T<sub>H</sub>17 subpopulation are T-cells that release mainly IL-17, which has both neutrophil chemotactic activity and the potential to promote autoimmunity.



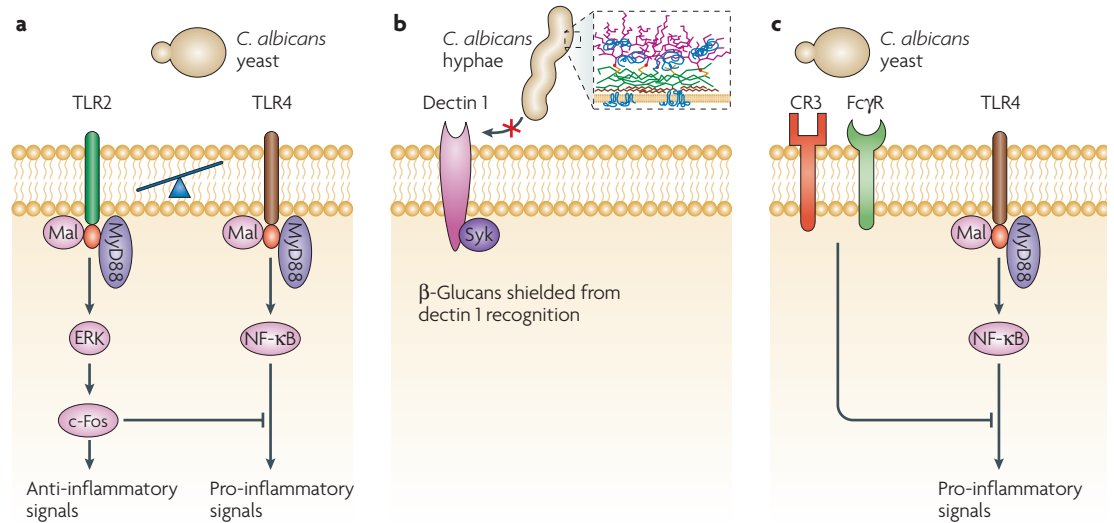
**Figure 3 | Recognition of *Candida albicans* at the membrane level.** Recognition of *C. albicans* at the level of the cell membrane is mediated by TLRs and LRs. TLR4 induces mainly pro-inflammatory signals in monocytic cell types (monocytes, macrophages and DCs) through the MyD88–Mal-mediated NF-κB and MAPK pathways, while stimulating T<sub>H</sub>1 responses through IRF3-dependent mechanisms mainly in plasmacytoid DCs. TLR2 stimulates the production of moderate amounts of pro-inflammatory cytokines and strong IL-10 and TGFβ responses. On the one hand, this leads to the induction of a tolerant phenotype in DCs, through an ERK/MAPK-dependent mechanism<sup>121</sup>. On the other hand, in monocytes and macrophages it induces TGFβ and IL-10, and subsequent proliferation of T<sub>Reg</sub> cells and immunosuppression<sup>78</sup>. The pro-inflammatory effects of TLR2 can be amplified by dectin 1 and galectin 3 — the latter especially in macrophages. In addition to amplifying the effects of TLR2, the non-classical lectin-like receptor dectin 1 induces IL-2, IL-10 and T<sub>H</sub>17 responses through a Syk–CARD9 cascade, independently of its interaction with TLR2. The classical lectin-like receptor, the MR, induces pro-inflammatory effects in monocytes and macrophages, whereas chitin-dependent stimulation of these cells induces mainly T<sub>H</sub>2 responses<sup>122</sup>, although this effect has yet to be demonstrated for *C. albicans*, and the identity of the chitin receptor is unknown. Other less well characterized pathways include stimulation of TNF and IL-1Ra by dectin 2, while engagement of DC-SIGN in DCs induces production of the immunosuppressive cytokine IL-10. CARD9, caspase recruitment domain protein 9; DC, dendritic cell; ERK, extracellular signal related kinase; FcγR, Fcγ receptor; IL, interleukin; IL-1Ra, interleukin-1 receptor antagonist; IFNγ, interferon-γ; IRF3, interferon regulatory factor 3; JNK, Jun N-terminal kinase; LR, lectin receptor; MAPK, mitogen-activated protein kinase; MR, mannose receptor; MyD88, myeloid differentiation primary response gene 88; NF-κB, nuclear factor κB; PLM, phospholipomannan; Syk, spleen tyrosine kinase; TGFβ, transforming growth factor-β; T<sub>H</sub>, T helper; TLR, Toll-like receptor; TNF, tumour necrosis factor; T<sub>Reg</sub>, regulatory T-cell.

have observed variable results, with TLR4<sup>-/-</sup> mice being more susceptible in models of intragastric infection or intravenous re-infection<sup>55</sup>, not different from wild-type animals in models of intravenous infection with *C. albicans* yeast<sup>104</sup>, or even surviving longer in a model of intravenous infection with *C. albicans* hyphae<sup>55</sup>. The differences between the experimental models and/or the *C. albicans* strains used are thought to be responsible for these differences.

Although no increased susceptibility of TLR9<sup>-/-</sup> mice to disseminated candidiasis has been observed, the fungal burden in the organs of TLR9-deficient animals tends to be lower than in control mice<sup>55</sup>. Although pro-inflammatory cytokine production was slightly lower in TLR9<sup>-/-</sup> macrophages that had been stimulated with *C. albicans*, this mild difference did not result in

a deleterious outcome of infection, probably owing to compensatory pathways that are mediated either by other TLRs or by LR-dependent mechanisms.

The study of the role of LRs in the *in vivo* response to *C. albicans* infections is still in its infancy. Recently, it has been shown that dectin 1<sup>-/-</sup> mice are more susceptible to *C. albicans* infection, and infection results in increased fungal growth in the organs and accelerated death<sup>105</sup>. This is consistent with the increased susceptibility of CARD9<sup>-/-</sup> mice to disseminated candidiasis<sup>89</sup>. Notably, all of the major components of antifungal defence — cytokine release, phagocyte recruitment, phagocytosis and microbial killing — are impaired in the knockout mice, which suggests an important role for dectin-1-mediated recognition of β-glucans for anti-candidal defence *in vivo*<sup>105</sup>. However, these results are at odds with



**Figure 4 | *Candida albicans* mechanisms to escape the innate response using pattern-recognition receptors.** **a** | The balance between the signals that are induced by Toll-like receptor 2 (TLR2) and TLR4 seems to have a crucial role in the regulation of the immune response. TLR2-mediated signals are mainly anti-inflammatory and can be deleterious when activated prematurely or excessively, TLR4-mediated signals are mainly pro-inflammatory. **b** | Shielding of the  $\beta$ -glucans by mannans in hyphae prevents activation of the dectin 1 signalling pathways. **c** | Inhibitory signals from complement receptor 3 (CR3) and Fc $\gamma$  receptor II (Fc $\gamma$ RII)/Fc $\gamma$ RIII have inhibitory effects on the activation of the immune system via TLR4. ERK, extracellular signal related kinase; MyD88, myeloid differentiation primary response gene 88; NF- $\kappa$ B, nuclear factor  $\kappa$ B; Syk, spleen tyrosine kinase, TLR, Toll-like receptor.

another study in dectin 1<sup>-/-</sup> mice, which failed to discern increased susceptibility to candidiasis<sup>106</sup>. Differences in the experimental host or between different *C. albicans* strains might account for these discrepancies, and additional studies are needed to pinpoint the origin of these differences.

Studies on the *in vivo* roles of other LRs in the response to *C. albicans* defences are also scarce. In one study, intraperitoneal administration of *C. albicans* resulted in an increased fungal burden in the organs of MR<sup>-/-</sup> mice, compared to controls, although survival was not affected<sup>107</sup>. However, intraperitoneal injection of *C. albicans* is not an established experimental model of invasive candidiasis, as it mainly induces peritonitis rather than a disseminated infection<sup>108</sup>. A recent study has demonstrated an important role for galectin 3 in the recognition of *C. albicans* in the gut<sup>29</sup>, with  $\beta$ -(1,2)-mannosides able to block colonization and invasion of *C. albicans* in the intestines<sup>9</sup>. Finally, like mice defective in TLR2, mice defective in CR3 or Fc $\gamma$ R are more resistant to disseminated candidiasis, and display more vigorous cytokine release and antifungal killing, in line with the immunosuppressive effect of these receptor pathways on the inflammatory response<sup>60</sup>.

It has become clear that PRRs are important for the host response to *C. albicans*, with various TLRs and LRs having distinctive roles in innate immunity: some receptors have a more pro-inflammatory role (for example, TLR4, dectin 1 and the MR), whereas others exert immunosuppressive effects (for example, TLR2, CR3 and Fc $\gamma$ R) (FIGS 3,4). In addition, the choice of experimental model and the *C. albicans* strain used might have an important impact on the outcome of infection. More work must

still be done to fully understand the role of PRRs in the various types of *C. albicans* infections *in vivo*. Studies of disseminated candidiasis are more advanced, but little work has been done in other forms of *C. albicans* infection, such as oropharyngeal or vaginal candidiasis, and little attention has been paid to the mechanisms that lead to colonization rather than infection.

**Escape mechanisms based on PRRs**

As evidence supporting the important role of PRRs in the recognition of *C. albicans* for the induction of an immune response accumulates, data have also emerged to show that certain fungal pathogens have evolved strategies to curtail recognition by these receptors, or to use it to their own advantage to evade the host response. For example, it was recently shown in *Drosophila melanogaster* that Gram-negative binding protein 3 (GNBP3) acts as a dectin-1-like lectin-recognition mechanism for fungal glucans, including that of *C. albicans*<sup>109</sup>. Certain fungal insect pathogens secrete proteases that degrade GNBP3 to avoid detection, but the *D. melanogaster* persophone protease is activated by the fungal protease virulence factor, which results in the bypass of GNBP3 and the activation of the Toll pathway downstream of the PRR. It remains to be seen whether there is a resonance of this co-evolution between recognition mechanisms and virulence factors in human–fungal interactions.

In the mammalian host, inappropriate or premature activation of immunomodulatory receptors can be used as an escape route by *C. albicans*, as detailed above for the outcome of systemic candidiasis in TLR2<sup>-/-</sup> mice (FIG. 4a). Modulation of DC function can also be a target



for escape mechanisms, with the MR and DC-SIGN possibly inducing anti-inflammatory cytokine production and immunosuppression<sup>110,111</sup>. Thus it seems that the MR might have a dual role, either pro-inflammatory (in monocytes and macrophages) or immunosuppressive (in DCs), although this assumption is still controversial. In addition, the recognition of  $\beta$ -glucans by CR3 does not trigger protective host responses, such as the respiratory burst<sup>42</sup>, and can repress pro-inflammatory signals<sup>44</sup> (FIG. 4). In line with these immunosuppressive effects *in vitro*, CR3-knockout mice are more resistant to disseminated candidiasis<sup>60</sup>, and are also more resistant to *Blastomyces dermatitidis* infection<sup>43</sup>. Some studies have suggested that the differential activation of stimulatory and inhibitory receptors is caused by the dimorphic nature of *C. albicans* yeast and hyphae, which induce different DC activation profiles. The recognition of yeasts by DCs is mediated mainly by the MR, dectin 1 (REF. 60) and DC-SIGN<sup>27,112</sup>, and hyphae are recognized mainly through dectin 2, CR3, Fc $\gamma$ RII and Fc $\gamma$ RIII<sup>60</sup>.

In addition to inducing direct anti-inflammatory effects, *C. albicans* has developed strategies to either block or avoid recognition by stimulatory PRRs (FIG. 4b). The morphogenetic change of *C. albicans* from a yeast to a hyphal form, after adhesion of yeast cells to the intravascular endothelium and invasion of the surrounding tissue, might represent a mechanism whereby the pathogen can avoid recognition of  $\beta$ -glucans by the immune system<sup>113</sup>. Hyphal filaments lack surface-exposed  $\beta$ -glucan and have a range of other cell-surface modifications that could influence immune detection<sup>11</sup>. In addition, the induction of the pro-inflammatory response through dectin 1 can be prevented, because the mannoprotein layer covers the  $\beta$ -glucan layer of the cell wall, thus blocking the  $\beta$ -glucan–dectin 1 interaction<sup>11</sup>. Accordingly, exposure of  $\beta$ -glucan through treatment with the antifungal caspofungin, by heat treatment of cells or by mutation of glycosylation genes that result in depletion of surface mannan all led to an enhanced dectin-1-mediated pro-inflammatory cytokine signal. This suggests that the mannoprotein layer is a mask that downregulates the pro-inflammatory dectin-1-mediated response<sup>114</sup>. As the exposed  $\beta$ -glucan of intact *C. albicans* yeast cells is thought to be presented mainly at the surface of the wall as bud scars, the attenuation of dectin-1-mediated recognition during germ tube evagination could be due to the expansion of the hyphal cell surface that lacks any bud scars. In line with this, Rappleye *et al.* showed that the  $\beta$ -glucan–dectin 1 interaction between *Histoplasma capsulatum* and macrophages can be shielded by a different cell wall component —  $\alpha$ -(1,3)-glucan<sup>115</sup>. The presence of  $\alpha$ -(1,3)-glucan in yeast cells prevents recognition through dectin 1, and mutation of *AGS1* (which encodes  $\alpha$ -(1,3)-glucan synthase) results in greatly enhanced TNF $\alpha$  production<sup>115</sup>. Therefore, both mannans and  $\alpha$ -glycans can act as shields that mask dectin-1-mediated immune responses.

It is important to note that mannans are in themselves immunostimulatory<sup>15,24,116–118</sup>. How can the immunostimulatory properties of mannans be reconciled with the apparent inhibitory effect of masking

the  $\beta$ -glucan–dectin 1 interactions? The answer to this paradox might reside in the complex interaction between TLRs and LRs. Although both mannans and glucans can induce pro-inflammatory signals, concomitant stimulation of the mannan–TLR and  $\beta$ -glucan–dectin 1 pathways has a synergistic effect on the amplification of the immune response. Amplification mechanisms between dectin 1 and TLR2 (REFS 49,50), and between dectin 1 and TLR4 (K. M. Dennehy and colleagues, unpublished observations) have recently been documented. If the interaction of  $\beta$ -glucans with dectin 1 is shielded by mannans, this synergism may be lost, with inefficient activation of host defence, despite recognition of mannans. It is therefore mainly the loss of synergistic effects, rather than the inhibition of an individual recognition pathway, that may lead to defective cytokine release.

### An integrated model of innate recognition

Progress in our understanding of the mechanisms responsible for *C. albicans* recognition, as described above, allows us to suggest an integrated view of how *C. albicans* is recognized and of how the host innate immune response is activated during candidiasis. Although we are still in the early stages of understanding the complexity of fungal recognition, and there is still much to learn, there are several principles that characterize recognition of *C. albicans*.

- First, recognition depends on ‘tasting’ several PAMPs in the fungal cell wall. Specific receptor systems have evolved for the recognition of the major polysaccharide cell wall components, such as the MR and DC-SIGN for the recognition of branched *N*-linked mannan, TLR4 for linear *O*-linked mannan, galectin 3 for  $\beta$ -mannosides, dectin 1 and TLR2 for  $\beta$ -glucan and PLM, and CR3 for  $\beta$ -(1,6)-glucan.
- Second, despite overlapping and sometimes redundant functions, each ligand–receptor system activates specific intracellular signalling pathways, and this has distinct consequences for the activation of the various components of the host immune response.
- Third, differential expression of the various PRRs is an important mechanism for the cell-type-specific response to fungal pathogens (FIG. 2).
- Fourth, the fully integrated response to a specific pathogen depends on the mosaic of PRRs and receptor complexes that are engaged. Co-stimulation via multiple PAMP–PRR interactions can increase both the sensitivity and the specificity of the immune recognition process.

These four principles can be extrapolated to immune recognition of any microorganism. For example, the final response upon stimulation of monocytes by *C. albicans* depends on integrating the signals that are received from TLR2, dectin 1, TLR4 and the MR. This response differs from the stimulation that is induced by the Gram-negative bacterium *Escherichia coli*, which can be ascribed to recognition of lipopolysaccharide by TLR4, membrane lipopeptides by TLR2 and flagellin by TLR5. By contrast, Gram-positive bacteria are mainly recognized by TLR2 (which recognizes lipoteichoic acid) and NOD2 (which recognizes peptidoglycans). The different

intracellular events that are stimulated by these pathways are ultimately responsible for the tailored innate response to infection by different classes of microorganisms.

From this perspective, although described here for *C. albicans*, these general principles can be considered as a blueprint for pattern recognition of all pathogenic microorganisms by the innate immune response.

**Future directions**

In parallel with the general renaissance in the field of innate immunity, our understanding of *C. albicans* recognition by the innate immune system has improved considerably over the past decade. Despite this progress, more challenges lie ahead. Although we have gained a much better understanding of the basal (polysaccharide) structures that are recognized by the PRRs, more work must be done to understand the details of the interactions of these PAMPs with their receptors on the host cell membrane. Recent evidence, for example, demonstrates that dectin 1 associates with tetraspanins, which suggests that dectin 1 forms receptor complexes on the cell surface that might be involved in regulating immune responses<sup>88</sup>. In addition, little is known about the recognition of the other components of the *C. albicans* cell wall, such as the proteins themselves, or the possible sensing of products that are secreted by *C. albicans*. The differential recognition of the yeast and hyphal forms also promises to be a fertile area for future research.

An additional challenge is to provide evidence that the pattern-recognition pathways identified in experimental models are also applicable during *C. albicans* infections in humans. This challenge can be partly met by experiments

in which human primary cells are exposed to *C. albicans*. However, valuable information can also be gathered from immunogenetic studies. For example, TLR4 polymorphisms have recently been identified as a susceptibility trait for systemic candidiasis, which supports a role for this receptor in the host immune response to *C. albicans*<sup>119</sup>. Studies of additional functional polymorphisms in larger cohorts of patients are needed to confirm the role of the various pathways in the pathogenesis of candidiasis

The ultimate ambition of research into the biology of host–fungus interactions is to be able to translate findings at the molecular level of the interactions into new treatments for patients who are vulnerable to lethal fungal infections. Although direct blockade or stimulation of PRRs might be problematic as a viable therapeutic approach, vaccine biology has become a domain in which our newly acquired knowledge of PRRs is already demonstrating potential<sup>120</sup>. The understanding of the modulation of DC function by PRRs has led to the design of novel and specific vaccine adjuvants, and this work is likely to have a major impact on the development of future antifungal vaccines.

In conclusion, in this Review we have presented an integrated model of innate pattern recognition of an important human pathogen, the fungus *C. albicans*, including a description of the PAMPs of *C. albicans* that are recognized by the host, to the receptor pathways that are stimulated by specific fungal molecular structures. We have also discussed and speculated on how these signals are integrated to bring about efficient activation of the host innate response. This model, which focuses on the interaction of *C. albicans* with host defences, could be conceptually pertinent to the modelling of various other host–pathogen interactions.

1. Edmond, M. B. *et al.* Nosocomial bloodstream infections in United States hospitals: a three-year analysis. *Clin. Infect. Dis.* **29**, 239–244 (1999).
2. Enoch, D. A., Ludlam, H. A. & Brown, N. M. Invasive fungal infections: a review of epidemiology and management options. *J. Med. Microbiol.* **55**, 809–818 (2006).
3. Wisplinghoff, H. *et al.* Nosocomial bloodstream infections in US hospitals: analysis of 24,179 cases from a prospective nationwide surveillance study. *Clin. Infect. Dis.* **39**, 309–317 (2004).
4. Gudlaugsson, O. *et al.* Attributable mortality of nosocomial candidemia, revisited. *Clin. Infect. Dis.* **37**, 1172–1177 (2003).
5. Stevens, D. A. Combination immunotherapy and antifungal chemotherapy. *Clin. Infect. Dis.* **26**, 1266–1269 (1998).
6. Mitchison, A. Will we survive? *Sci. Am.* **269**, 136–144 (1993).
7. Hoebe, K., Janssen, E. & Beutler, B. The interface between innate and adaptive immunity. *Nature Immunol.* **5**, 971–974 (2004).
8. Ernst, J. F. & Prill, S. K. O-glycosylation. *Med. Mycol.* **39**, 1, 67–74 (2001).
9. Cutler, J. E. N-glycosylation of yeast, with emphasis on *Candida albicans*. *Med. Mycol.* **39**, 75–86 (2001).
10. Klis, F. M., Boorsma, A. & De Groot, P. W. Cell wall construction in *Saccharomyces cerevisiae*. *Yeast* **23**, 185–202 (2006).
11. Gantner, B. N., Simmons, R. M. & Underhill, D. M. Dectin-1 mediates macrophage recognition of *Candida albicans* yeasts but not filaments. *EMBO J.* **24**, 1277–1286 (2005).  
**Describes the differential recognition of *C. albicans* yeast and hyphae by dectin 1 as a major escape mechanism.**
12. Suzuki, A. in *Candida and Candidosis* (ed. Calderone, R. A.) 29–36 (ASM Press, Washington, 2002).
13. Vecchiarelli, A., Puliti, M., Torosantucci, A., Cassone, A. & Bistoni, F. *In vitro* production of tumor necrosis factor by murine splenic macrophages stimulated with mannoprotein constituents of *Candida albicans* cell wall. *Cell. Immunol.* **134**, 65–76 (1991).
14. Garner, R. E., Rubanowicz, K., Sawyer, R. T. & Hudson, J. A. Secretion of TNF- $\alpha$  by alveolar macrophages in response to *Candida albicans* mannan. *J. Leuk. Biol.* **55**, 161–168 (1994).
15. Pietrella, D., Bistoni, G., Corbucci, C., Perito, S. & Vecchiarelli, A. *Candida albicans* mannoprotein influences the biological function of dendritic cells. *Cell. Microbiol.* **8**, 602–612 (2006).
16. Gomez, M. J. *et al.* Purification and biochemical characterization of a 65-kilodalton mannoprotein (MP65), a main target of anti-*Candida* cell-mediated immune responses in humans. *Infect. Immun.* **64**, 2577–2584 (1996).
17. Gomez, M. J. *et al.* Biochemical and immunological characterization of MP65, a major mannoprotein antigen of the opportunistic human pathogen *Candida albicans*. *Infect. Immun.* **68**, 694–701 (2000).
18. Mencacci, A. *et al.* A mannoprotein constituent of *Candida albicans* that elicits different levels of delayed-type hypersensitivity, cytokine production, and anticandidal protection in mice. *Infect. Immun.* **62**, 5353–5360 (1994).
19. Wileman, T. E., Lennartz, M. R. & Stahl, P. D. Identification of the macrophage mannose receptor as a 175-kDa membrane protein. *Proc. Natl Acad. Sci. USA* **83**, 2501–2505 (1986).
20. Stephenson, J. D. & Shepherd, V. L. Purification of the human alveolar macrophage mannose receptor. *Biochem. Biophys. Res. Commun.* **148**, 883–889 (1987).
21. Stahl, P. D., Rodman, J. S., Miller, M. J. & Schlesinger, P. H. Evidence for receptor-mediated binding of glycoproteins, glycoconjugates, and lysosomal glycosidases by alveolar macrophages. *Proc. Natl Acad. Sci. USA* **75**, 1399–1403 (1978).
22. Linehan, S. A., Martinez-Pomares, L. & Gordon, S. Macrophage lectins in host defence. *Microbes Infect.* **2**, 279–288 (2000).
23. Kery, V., Krepinsky, J. J., Warren, C. D., Capek, P. & Stahl, P. D. Ligand recognition by purified human mannose receptor. *Arch. Biochem. Biophys.* **298**, 49–55 (1992).
24. Netea, M. G. *et al.* Immune sensing of *Candida albicans* requires cooperative recognition of mannans and glucans by lectin and Toll-like receptors. *J. Clin. Invest.* **116**, 1642–1650 (2006).  
**Proposed, for the first time, an integrated model of fungal recognition.**
25. Tada, H. *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**, 503–512 (2002).
26. van der Graaf, C. A. A., Netea, M. G., Verschueren, I., van der Meer, J. W. M. & Kullberg, B. J. Differential cytokine production and Toll-like receptor signaling pathways by *Candida albicans* blastoconidia and hyphae. *Infect. Immun.* **73**, 7458–7464 (2005).
27. Cambi, A. *et al.* The C-type lectin DC-SIGN (CD209) is an antigen-uptake receptor for *Candida albicans* on dendritic cells. *Eur. J. Immunol.* **33**, 532–538 (2003).
28. Jouault, T. *et al.* *Candida albicans* phospholipomannan is sensed through Toll-like receptors. *J. Infect. Dis.* **188**, 165–172 (2003).
29. Jouault, T. *et al.* Specific recognition of *Candida albicans* by macrophages requires galectin-3 to discriminate *Saccharomyces cerevisiae* and needs association with TLR2 for signaling. *J. Immunol.* **177**, 4679–4687 (2006).

30. Ariuzumi, K. *et al.* Cloning of a second dendritic cell-associated C-type lectin (dectin-2) and its alternatively spliced isoforms. *J. Biol. Chem.* **275**, 11957–11963 (2000).
31. Taylor, P. R. *et al.* Dectin-2 is predominantly myeloid restricted and exhibits unique activation-dependent expression on maturing inflammatory monocytes elicited *in vivo*. *Eur. J. Immunol.* **35**, 2163–2174 (2005).
32. Sato, K. *et al.* 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.* **281**, 38854–38866 (2006).
33. McGreal, E. P. *et al.* The carbohydrate-recognition domain of Dectin-2 is a C-type lectin with specificity for high mannose. *Glycobiology* **16**, 422–430 (2006).
34. Klis, F. M., de Groot, P. & Hellingwerf, K. Molecular organization of the cell wall of *Candida albicans*. *Med. Mycol.* **39**, 1–8 (2001).
- A model of the C. albicans cell wall.**
35. Torosantucci, A. *et al.* A novel glyco-conjugate vaccine against fungal pathogens. *J. Exp. Med.* **202**, 597–606 (2005).
36. Brown, G. D. & Gordon, S. Immune recognition of fungal  $\beta$ -glucans. *Cell. Microbiol.* **7**, 471–479 (2005).
37. Obayashi, T. *et al.* Plasma (1,3)- $\beta$ -D-glucan measurement in diagnosis of invasive deep mycosis and fungal febrile episodes. *Lancet* **345**, 17–20 (1995).
38. Thornton, B. P., Vetricka, V., Pitman, M., Goldman, R. C. & Ross, G. D. Analysis of the sugar specificity and molecular location of the  $\beta$ -glucan-binding lectin site of complement receptor type 3 (CD11b/CD18). *J. Immunol.* **156**, 1235–1246 (1996).
39. Diamond, M. S., Garcia-Aguilar, J., Bickford, J. K., Corbi, A. L. & Springer, T. A. The I domain is a major recognition site on the leukocyte integrin Mac-1 (CD11b/CD18) for four distinct adhesion ligands. *J. Cell Biol.* **120**, 1031–1043 (1993).
40. Forsyth, C. B. & Mathews, H. L. Lymphocyte adhesion to *Candida albicans*. *Infect. Immun.* **70**, 517–527 (2002).
41. Forsyth, C. B., Plow, E. F. & Zhang, L. Interaction of the fungal pathogen *Candida albicans* with integrin CD11b/CD18: recognition by the I domain is modulated by the lectin-like domain and the CD18 subunit. *J. Immunol.* **161**, 6198–6205 (1998).
42. Wright, S. D. & Silverstein, S. C. Receptors for C3b and C3bi promote phagocytosis but not the release of toxic oxygen from human phagocytes. *J. Exp. Med.* **158**, 2016–2023 (1983).
43. Brandhorst, T. T., Wuthrich, M., Finkel-Jimenez, B., Warner, T. & Klein, B. S. Exploiting type 3 complement receptor for TNF- $\alpha$  suppression, immune evasion, and progressive pulmonary fungal infection. *J. Immunol.* **173**, 7444–7453 (2004).
44. Wright, S. D. & Silverstein, S. C. Tumor-promoting phorbol esters stimulate C3b and C3bi receptor-mediated phagocytosis in cultured human monocytes. *J. Exp. Med.* **156**, 1149–1164 (1982).
45. Brown, G. D. & Gordon, S. Fungal  $\beta$ -glucans and mammalian immunity. *Immunity* **19**, 311–315 (2003).
- A review that describes the role of  $\beta$ -glucan recognition in host defence.**
46. Rogers, N. C. *et al.* SYK-dependent cytokine induction by dectin-1 reveals a novel pattern recognition pathway for C type lectins. *Immunity* **22**, 507–517 (2005).
47. Gow, N. A. R. *et al.* 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.* (in the press).
48. Leibundgut-Landmann, S. *et al.* SYK- and CARD9-dependent coupling of innate immunity to the induction of T helper cells that produce interleukin 17. *Nature Immunol.* **8**, 630–638 (2007).
- This study demonstrates the specific activation of  $T_H17$  cells by dectin 1.**
49. Brown, G. D. *et al.* Dectin-1 mediates the biological effects of  $\beta$ -glucans. *J. Exp. Med.* **197**, 1119–1124 (2003).
50. Gantner, B. N., Simmons, R. M., Canavera, S. J., Akira, S. & Underhill, D. M. Collaborative induction of inflammatory responses by dectin-1 and Toll-like receptor 2. *J. Exp. Med.* **197**, 1107–1117 (2003).
- References 49 and 50 show for the first time the collaboration between TLRs (TLR2) and LRs (dectin 1).**
51. Rubin-Bejerano, I., Abejón, C., Magnelli, P., Grisafi, P. & Fink, G. R. Phagocytosis by human neutrophils is stimulated by a unique fungal cell wall component. *Cell Host Microbe* **2**, 55–67 (2007).
52. Minke, R. & Blackwell, J. The structure of  $\alpha$ -chitin. *J. Mol. Biol.* **120**, 167–181 (1978).
53. van der Graaf, C. A. *et al.* Nucleotide oligomerization domain 2 (Nod2) is not involved in the pattern recognition of *Candida albicans*. *Clin. Vaccine Immunol.* **13**, 423–425 (2006).
54. Wagner, H. Toll meets bacterial CpG-DNA. *Immunity* **14**, 499–502 (2001).
55. Bellocchio, S. *et al.* The contribution of Toll-like/IL-1 receptor superfamily to innate and adaptive immunity to fungal pathogens *in vivo*. *J. Immunol.* **172**, 3059–3069 (2004).
56. Kozel, T. R. Activation of the complement system by pathogenic fungi. *Clin. Microbiol. Rev.* **9**, 34–46 (1996).
57. Kozel, T. R., Weinhold, L. C. & Lupan, D. M. Distinct characteristics of initiation of the classical and alternative complement pathways by *Candida albicans*. *Infect. Immun.* **64**, 3360–3368 (1996).
58. Herre, J. *et al.* Dectin-1 uses novel mechanisms for yeast phagocytosis in macrophages. *Blood* **104**, 4038–4045 (2004).
59. Ezekowitz, R. A. B. *et al.* Restoration of phagocyte function by interferon- $\gamma$  in X-linked chronic granulomatous disease occurs at the level of a progenitor cell. *Blood* **76**, 2443–2448 (1990).
60. Romani, L. *et al.* The exploitation of distinct recognition receptors in dendritic cells determines the full range of host immune relationships with *Candida albicans*. *Int. Immunol.* **16**, 149–161 (2004).
61. Le Cabec, V., Emorine, L. J., Toesca, I., Cougoule, C. & Maridonneau-Parini, I. The human macrophage mannose receptor is not a professional phagocytic receptor. *J. Leuk. Biol.* **77**, 934–943 (2005).
62. Blander, J. M. & Medzhitov, R. Toll-dependent selection of microbial antigens for presentation by dendritic cells. *Nature* **440**, 808–812 (2006).
63. Romani, L. Immunity to fungal infections. *Nature Rev. Immunol.* **4**, 1–13 (2004).
64. Kennedy, A. D. *et al.* Dectin-1 promotes fungicidal activity of human neutrophils. *Eur. J. Immunol.* **37**, 467–478 (2007).
65. Aratani, Y. *et al.* Severe impairment in early host defense against *Candida albicans* in mice deficient in myeloperoxidase. *Infect. Immun.* **67**, 1828–1836 (1999).
66. Mansour, M. K. & Levitz, S. M. Interactions of fungi with phagocytes. *Curr. Opin. Microbiol.* **5**, 359–365 (2002).
67. Reeves, E. P. *et al.* Killing activity of neutrophils is mediated through activation of proteases by  $K^+$  flux. *Nature* **416**, 291–297 (2002).
68. Kohatsu, L., Hsu, D. K., Jegalian, A. G., Liu, F. T. & Baum, L. G. Galectin-3 induces death of *Candida* species expressing specific  $\beta$ -1, 2-linked mannans. *J. Immunol.* **177**, 4718–4726 (2006).
69. Akira, S. & Hemmi, H. Recognition of pathogen-associated molecular patterns by TLR family. *Immunol. Letters* **85**, 85–95 (2003).
70. Toshchakov, V. *et al.* TLR4, but not TLR2, mediates IFN $\beta$ -induced STAT1  $\alpha/\beta$ -dependent gene expression in macrophages. *Nature Immunol.* **3**, 392–398 (2002).
71. Netea, M. G. *et al.* The role of Toll-like receptors in the defense against disseminated candidiasis. *J. Infect. Dis.* **185**, 1483–1489 (2002).
- Demonstrates, for the first time, the role of TLRs in C. albicans recognition.**
72. Hirschfeld, M. *et al.* Signaling by Toll-like receptor 2 and 4 agonists results in differential gene expression in murine macrophages. *Infect. Immun.* **69**, 1477–1482 (2001).
73. Re, F. & Strominger, J. L. Toll-like receptor 2 (TLR2) and TLR4 differentially activate human dendritic cells. *J. Biol. Chem.* **276**, 37692–37699 (2001).
74. Agrawal, S. *et al.* 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.* **171**, 4984–4989 (2003).
75. Dillon, S. *et al.* Yeast zymosan, a stimulus for TLR2 and dectin-1, induces regulatory antigen-presenting cells and immunological tolerance. *J. Clin. Invest.* **116**, 916–928 (2006).
76. Pulendran, B. *et al.* Lipopolysaccharides from distinct pathogens induce different classes of immune responses *in vivo*. *J. Immunol.* **167**, 5067–5076 (2001).
77. Netea, M. G. *et al.* Toll-like receptor 2 inhibits cellular responses against *Candida albicans* through pathways mediated by IL-10 and CD4 $^+$  CD25 $^+$  regulatory T cells. *J. Immunol.* **172**, 3712–3718 (2004).
- Demonstrates, for the first time, induction of  $T_{Reg}$  cells through TLR2 signals.**
78. Suttmüller, R. P. *et al.* Toll-like receptor 2 controls expansion and function of regulatory T cells. *J. Clin. Invest.* **116**, 485–494 (2006).
79. Van der Kleij, D. *et al.* A novel host-parasite lipid cross-talk: schistosomal lyso-phosphatidylserine activates Toll-like receptor 2 and affects immune polarization. *J. Biol. Chem.* **277**, 48122–48129 (2002).
80. Diterich, I., Rauter, C., Kirschning, C. J. & Hartung, T. *Borrelia burgdorferi*-induced tolerance as a model of persistence via immunosuppression. *Infect. Immun.* **71**, 3979–3985 (2003).
81. Underhill, D. M. *et al.* The Toll-like receptor 2 is recruited to macrophage phagosomes and discriminates between pathogens. *Nature* **401**, 811–815 (1999).
- One of the first studies to demonstrate the discrimination of pathogens by TLRs.**
82. Netea, M. G., van de Veerdonk, F., Verschuere, I., van der Meer, J. W. & Kullberg, B. J. Role of TLR1 and TLR6 in the host defense against disseminated candidiasis. *FEBS Immunol. Med. Microbiol.* (in the press).
83. Brown, G. R., Silva, M. D., Thompson, P. A. & Beutler, B. Lymphoid hyperplasia, CD45RB $^{high}$  to CD45RB $^{low}$  T-cell imbalance, and suppression of type I diabetes mellitus result from TNF blockade in NOD-NOD-*scid* adoptive T cell transfer. *Diabetologia* **41**, 1502–1510 (1998).
84. Steele, C. *et al.* The  $\beta$ -glucan receptor dectin-1 recognizes specific morphologies of *Aspergillus fumigatus*. *PLoS Pathog.* **4**, 323–334 (2005).
85. Steele, C. *et al.* Alveolar macrophage-mediated killing of *Pneumocystis carinii* f. sp. *muris* involves molecular recognition by the Dectin-1  $\beta$ -glucan receptor. *J. Exp. Med.* **198**, 1677–1688 (2003).
86. Viriyakosol, S., Fierer, J., Brown, G. D. & Kirkland, T. N. Innate immunity to the pathogenic fungus *Coccidioides posadasii* is dependent on Toll-like receptor 2 and dectin-1. *Infect. Immun.* **73**, 1553–1560 (2005).
87. Slack, E. C. *et al.* Syk-dependent ERK activation regulates IL-2 and IL-10 production by DC stimulated with zymosan. *Eur. J. Immunol.* **37**, 1600–1612 (2007).
88. Meyer-Wentrup, F. *et al.* Dectin-1 interaction with tetraspanin CD37 inhibits IL-6 production. *J. Immunol.* **178**, 154–162 (2007).
89. Gross, O. *et al.* Card9 controls a non-TLR signalling pathway for innate anti-fungal immunity. *Nature* **442**, 651–656 (2006).
90. Acosta-Rodriguez, E. V. *et al.* Surface phenotype and antigenic specificity of human interleukin 17-producing T helper memory cells. *Nature Immunol.* **8**, 639–646 (2007).
91. Huang, W., Na, L., Fidel, P. L. & Schwarzenberger, P. Requirement for interleukin-17A for systemic anti-*Candida albicans* host defense in mice. *J. Infect. Dis.* **190**, 524–631 (2004).
92. Zelante, T. *et al.* IL-23 and the  $T_H17$  pathway promote inflammation and impair antifungal immune resistance. *Eur. J. Immunol.* **37**, 2695–2706 (2007).
93. Hsu, Y. M. *et al.* The adaptor protein CARD9 is required for innate immune responses to intracellular pathogens. *Nature Immunol.* **8**, 198–205 (2007).
94. Hara, H. *et al.* The adaptor protein CARD9 is essential for the activation of myeloid cells through ITAM-associated and Toll-like receptors. *Nature Immunol.* **8**, 619–629 (2007).
95. De Luca, A. *et al.* Functional yet balanced reactivity to *Candida albicans* requires TRIF, MyD88, and IDO-dependent inhibition of Rorc. *J. Immunol.* **179**, 5999–6008 (2007).
96. Weindl, G. *et al.* Human epithelial cells establish direct antifungal defense through TLR4-mediated signaling. *J. Clin. Invest.* (in the press).
97. Schaller, M. *et al.* Polymorphonuclear leukocytes (PMNs) induce protective Th1-type cytokine epithelial responses in an *in vitro* model of oral candidosis. *Microbiology* **150**, 2807–2813 (2004).

98. Fidel, P. L. Jr. History and update on host defense against vaginal candidiasis. *Am. J. Reprod. Immunol.* **57**, 2–12 (2007).
99. Pivarcsi, A. *et al.* Microbial compounds induce the expression of pro-inflammatory cytokines, chemokines and human  $\beta$ -defensin-2 in vaginal epithelial cells. *Microbes Infect.* **7**, 1117–1127 (2005).
100. Gozalbo, D., Roig, P., Villamon, E. & Gil, M. L. *Candida* and candidiasis: the cell wall as a potential molecular target for antifungal therapy. *Curr. Drug Targets Infect. Disord.* **4**, 117–135 (2004).
101. Marr, K. A. *et al.* Differential role of MyD88 in macrophage-mediated responses to opportunistic fungal pathogens. *Infect. Immun.* **71**, 5280–5286 (2003).
102. Villamon, E. *et al.* Toll-like receptor-2 is essential in murine defenses against *Candida albicans* infections. *Microbes Infect.* **6**, 1–7 (2004).
103. Blasi, E. *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.* **44**, 69–79 (2005).
104. Murciano, C. *et al.* 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.* **44**, 149–157 (2006).
105. Taylor, P. R. *et al.* Dectin-1 is required for  $\beta$ -glucan recognition and control of fungal infection. *Nature Immunol.* **8**, 31–38 (2007).
106. Saijo, S. *et al.* Dectin-1 is required for host defense against *Pneumocystis carinii* but not against *Candida albicans*. *Nature Immunol.* **8**, 39–46 (2007).
- References 105 and 106 are the first reports of *in vivo* fungal infections in dectin 1 knockout mice.**
107. Lee, S. J., Zheng, N. Y., Clavijo, M. & Nussenzweig, M. C. Normal host defense during systemic candidiasis in mannose receptor-deficient mice. *Infect. Immun.* **71**, 437–445 (2003).
108. Vonk, A. G., Netea, M. G., van Krieken, J. H., Van der Meer, J. W. M. & Kullberg, B. J. Delayed clearance of intraabdominal abscesses caused by *Candida albicans* in tumor necrosis factor- $\alpha$  and lymphotoxin- $\alpha$  deficient mice. *J. Infect. Dis.* **186**, 1815–1822 (2002).
109. Gottar, M. *et al.* Dual detection of fungal infections in *Drosophila* via recognition of glucans and sensing of virulence factors. *Cell* **127**, 1425–1437 (2006).
110. Geijtenbeek, T. B. *et al.* Mycobacteria target DC-SIGN to suppress dendritic cell function. *J. Exp. Med.* **197**, 7–17 (2003).
111. van Kooyk, Y., Engering, A., Lekkerkerker, A. N., Ludwig, I. S. & Geijtenbeek, T. B. Pathogens use carbohydrates to escape immunity induced by dendritic cells. *Curr. Opin. Immunol.* **16**, 488–493 (2004).
112. Taylor, P. R., Brown, G. D., Geldhof, A. B., Martinez-Pomares, L. & Gordon, S. Pattern recognition receptors and differentiation antigens define murine myeloid cell heterogeneity *ex vivo*. *Eur. J. Immunol.* **33**, 2090–2097 (2003).
113. d'Ostiani, C. F. *et al.* Dendritic cells discriminate between yeasts and hyphae of the fungus *Candida albicans*. Implications for initiation of T helper cell immunity *in vitro* and *in vivo*. *J. Exp. Med.* **191**, 1661–1674 (2000).
114. Wheeler, R. T. & Fink, G. R. A drug-sensitive genetic network masks fungi from the immune system. *PLoS Pathog.* **2**, 328–339 (2006).
115. Rappleye, C. A., Eissenberg, L. G. & Goldman, W. E. *Histoplasma capsulatum*  $\alpha$ -(1,3)-glucan blocks innate immune recognition by the  $\beta$ -glucan receptor. *Proc. Natl Acad. Sci. USA* **104**, 1366–1370 (2007).
116. Mangeney, M., Fischer, A., Le Deist, F., Latge, J. P. & Durandy, A. Direct activation of human B lymphocytes by *Candida albicans*-derived mannan antigen. *Cell. Immunol.* **122**, 329–337 (1989).
117. Lillegard, J. B., Sim, R. B., Thorkildson, P., Gates, M. A. & Kozel, T. R. Recognition of *Candida albicans* by mannan-binding lectin *in vitro* and *in vivo*. *J. Infect. Dis.* **193**, 1589–1597 (2006).
118. Sheng, K. C. *et al.* Mannan derivatives induce phenotypic and functional maturation of mouse dendritic cells. *Immunology* **118**, 372–383 (2006).
119. Van der Graaf, C. A. *et al.* Toll-like receptor 4 Asp299Gly/Thr399Ile polymorphisms are a risk factor for *Candida* bloodstream infection. *Eur. Cytokine Netw.* **17**, 29–34 (2006).
120. Cassone, A. & Torosantucci, A. Opportunistic fungi and fungal infections: the challenge of a single, general antifungal vaccine. *Expert Rev. Vaccines* **5**, 859–867 (2006).
121. Dillon, S. *et al.* Yeast zymosan, a stimulus for TLR2 and dectin-1, induces regulatory antigen-presenting cells and immunological tolerance. *J. Clin. Invest.* **116**, 916–928 (2006).
122. Reese, T. A. *et al.* Chitin induces accumulation in tissue of innate immune cells associated with allergy. *Nature* **447**, 92–96 (2007).

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## DATABASES

Entrez Genome project: <http://www.ncbi.nlm.nih.gov/sites/entrez?db=genomeprj>  
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