Original Articles

Variable recognition of Candida albicans strains by TLR4 and lectin recognition receptors

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The role of TLR4 in the recognition of Candida albicans has been brought into question. In order to assess whether discrepancies in the literature are due to differences in the recognition of various C. albicans strains, we selected 14 different isolates of C. albicans to evaluate their recognition by TLR4 and lectin receptors. We demonstrate that recognition of cell wall structures by lectin receptors is a consistent characteristic independent of the C. albicans strain selected, while recognition by TLR4 is a more variable feature. These data were corroborated by the increased susceptibility of TLR4-/- mice to a C. albicans strain recognized by TLR4, but not to a strain in which recognition has been shown to be independent of this receptor. This suggests a heavier reliance of in vivo antifungal host defense on lectin receptors than on TLRs, a notion compatible with the clinical picture in individuals deficient in MyD88/TLRs or dectin-1/CARD9.

Keywords Candida albicans, TLR4, lectin receptors, dectin-1, mannose receptor, cytokines

Introduction

Invasive fungal infections caused by Candida albicans are a serious threat to immunocompromised patients and those who undergo major surgical procedures, with mortality reaching 30–40% despite the availability of new classes of antifungal drugs [1,2]. Much has been done to elucidate the host defense mechanisms against systemic candidiasis. The innate host defense mechanisms leading to elimination of Candida during infection include the release of proinflammatory cytokines, contributing to the activation of phagocytosis and killing of the fungus by neutrophils and macrophages [3–5]. Proper stimulation of cytokine production, and in turn activation of host defense mechanisms, is dependent on the recognition of the invading pathogen by the innate immune system.

Recognition of microbial structures called pathogen-associated molecular patterns (PAMPs) by pattern-recognition receptors (PRRs) is essential for the effective activation of host defense mechanisms in general, and for cytokine production in particular. Earlier studies by our group and others have described the role of several PRRs for the recognition of components of the C. albicans cell wall. These include recognition of mannans and mannoproteins by mannose receptor (MR) and Toll-like receptor-4 (TLR4) [6–12], of phospholipomannan by TLR2 [13], and of β-glucans by dectin-1 [14–17]. In addition, several studies have demonstrated the important role of these receptors for host defense in experimental models of disseminated candidiasis. The TLR-adapter molecule MyD88 has been demonstrated to be involved in the resistance to disseminated candidiasis in mice [9,18], and individual receptors such as TLR2 [8,19] and...
dectin-1 [20] have been reported to modulate the defense against C. albicans infection.

TLR4 is also involved in the in vivo host defense against C. albicans. An initial study reported that TLR4-defective C3H/HeJ mice are more susceptible to disseminated candidiasis [7]. However, subsequent investigations have obtained variable results, with TLR4-/− mice being: (i) more susceptible in models of intragastric infection or intravenous reinfection [9], (ii) equally susceptible as wild-type animals in models of intravenous infection with Candida yeasts [21], or (iii) even showing longer survival in a lethal model of intravenous infection with C. albicans hyphae [9]. The reasons for these discrepancies have been unclear, although differences between the experimental models and/or the C. albicans strains were assumed to be responsible.

For this study, we hypothesized that most of these discrepancies are due to differences in the recognition of various C. albicans strains. In order to test this hypothesis, we selected 14 different strains of C. albicans based on their previously reported use in experimental models. The test isolates included representatives of 11 clades that were recently described based on multilocus sequence typing [22]. In the present study we investigated the recognition of a panel of C. albicans strains by TLR4 on the one hand, and by the lectin-like receptors dectin-1 and the macrophage mannose receptor (MR) on the other hand, in order to assess the variation of Candida recognition by pattern recognition receptors.

Materials and methods

Volunteers

Blood was collected from six healthy, nonsmoking volunteers who did not have any infectious or inflammatory disease. After obtaining informed consent, blood was collected by venipuncture into 10 ml EDTA syringes (Monoject).

Animals

TLR4-/− mice on a C57Bl/6J background (backcrossed for 10 generations) were kindly provided by Dr Shizuo Akira (Osaka University, Japan). In all experiments, knock-out mice and their control littermates (20–25 g, 6–8 weeks old) were used. The mice were fed sterilized laboratory chow (Hope Farms, Woerden, The Netherlands) and water ad libitum. The experiments were approved by the Ethics Committee on Animal Experiments of Radboud University Nijmegen.

C. albicans strains

A selection of C. albicans strains was used in the experiments. The C. albicans strains ATCC MYA-3573 (UC 820) [23], ATCC 18804 [24] and SCS 50350S [20] were chosen based on their wide use as described in the literature. An additional 11 C. albicans strains were selected to represent diverse clades described by multilocus sequence typing [22]. An overview of the strains used is presented in Table 1.

Table 1 Candida albicans strains used in the cytokine stimulation experiments.

<table>
<thead>
<tr>
<th>C. albicans strain</th>
<th>DST†</th>
<th>Clade</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>SCS B38619</td>
<td>567</td>
<td>6</td>
<td>[22]</td>
</tr>
<tr>
<td>SCS 154044</td>
<td>527</td>
<td>9</td>
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<tr>
<td>SCS 503505</td>
<td>596</td>
<td>12</td>
<td>[20]</td>
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<tr>
<td>SC 5314</td>
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<td>1</td>
<td>[22]</td>
</tr>
<tr>
<td>AM 20050372</td>
<td>756</td>
<td>8</td>
<td>[22]</td>
</tr>
<tr>
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<td>609</td>
<td>10</td>
<td>[22]</td>
</tr>
<tr>
<td>AM 20050370</td>
<td>754</td>
<td>11</td>
<td>[22]</td>
</tr>
<tr>
<td>ATCC 18804</td>
<td>ND</td>
<td>ND</td>
<td>[24]</td>
</tr>
<tr>
<td>J 990102</td>
<td>45</td>
<td>3</td>
<td>[22]</td>
</tr>
<tr>
<td>JIMS 103102</td>
<td>811</td>
<td>7</td>
<td>[22]</td>
</tr>
<tr>
<td>78/028</td>
<td>73</td>
<td>1</td>
<td>[22]</td>
</tr>
<tr>
<td>81/078</td>
<td>147</td>
<td>5</td>
<td>[22]</td>
</tr>
<tr>
<td>RV 4688</td>
<td>87</td>
<td>4</td>
<td>[22]</td>
</tr>
<tr>
<td>MYA 3573</td>
<td>ND</td>
<td>ND</td>
<td>[7]</td>
</tr>
</tbody>
</table>

ND – not determined.
†Diploid sequence type from MLST.

Induction of cytokine production

Separation and stimulation of peripheral blood mononuclear cells (PBMC) were performed as described previously [26]. Briefly, the PBMC fraction was obtained by density centrifugation of diluted blood (one part blood to one part pyrogen-free saline) over Ficoll-Paque (Pharmacia Biotech, Uppsala, Sweden). PBMC were washed twice in saline and suspended in culture medium (RPMI-1640 Dutch modification, ICN Biomedicals, Aurora, OH) [25]. C. albicans yeasts were killed by heating at 56°C for 1 hour.

References

Pattern recognition of Candida albicans

Subgroups of 5 animals were killed on days 3 and 7 of infection. To assess the tissue outgrowth of the microorganisms on these days, the kidneys of the sacrificed animals were removed aseptically, weighed, and homogenized in sterile saline in a tissue grinder. The number of viable Candida cells in the tissues was determined by plating serial dilutions on Sabouraud dextrose agar plates as previously described [28]. The CFU were counted after 24 h of incubation at 37°C, and expressed as log CFU/g tissue.

Cytokine assays

IL1β and TNF concentrations were measured by commercial ELISA kits (R&D systems, Minneapolis, MA), according to the instructions of the manufacturer.

C. albicans infection model

Two C. albicans strains were chosen for the in vivo experiments, i.e., strain SCS 503505 that has been shown to be recognized in vitro by TLR4, and the AM 2005/0370 strain that was not recognized by TLR4 in human PBMC. TLR4-/- mice and their control littersmates were injected intravenously with C. albicans (5 × 10⁴ CFU/mouse) in a 100 μl volume of sterile pyrogen-free phosphate-buffered saline (PBS). Subgroups of 5 animals were killed on days 3 and 7 of infection. To assess the tissue outgrowth of the microorganisms on these days, the kidneys of the sacrificed animals were removed aseptically, weighed, and homogenized in sterile saline in a tissue grinder. The number of viable Candida cells in the tissues was determined by plating serial dilutions on Sabouraud dextrose agar plates as previously described [28]. The CFU were counted after 24 h of incubation at 37°C, and expressed as log CFU/g tissue.

Statistical analysis

The differences between groups were analyzed by the Mann-Whitney U test, and were appropriate, by Kruskal-Wallis ANOVA. The level of significance between groups was set at p < 0.05. All experiments were performed at least twice, and the data are presented as cumulative results of all experiments performed. The screening data of the various strains are presented as a matrix (Fig. 1), in which the significant inhibitory effects of the various receptor blockers are depicted in red, while the lack of effect is depicted in green.

![Fig. 1](https://academic.oup.com/mmy/article-abstract/48/7/897/1054052)

Differential role of TLR4 and lectin receptors for Candida albicans recognition. PBMC isolated from six healthy volunteers were stimulated with various strains of C. albicans (1 × 10⁵/ml). TLR4 was blocked by the inhibitor Bartonella quintana LPS (20 μg/ml), dectin-1 by the inhibitor glucan-phosphate (10 μg/ml), and MR by mannan (200 μg/ml), before the stimulation with C. albicans. After 24 h incubation, the supernatants were collected and stored at –80°C until assayed by ELISA for IL-1β and TNF concentrations. The data after stimulation with the various strains are presented as a matrix, in which the significant inhibitory effects of the various receptor blockers are depicted in red, while the lack of effect is depicted in green.

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Results

Differential recognition of C. albicans strains

In order to assess the role of TLR4, dectin-1 and MR for the recognition of the various C. albicans strains, PBMC were preincubated with various specific inhibitors as indicated (see Fig. 1 and Table 2). Stimulation of the cells with the inhibitors alone did not influence cytokine production (not shown). Recognition of C. albicans by the lectin receptors dectin-1 and MR was a constant characteristic independent of the strain studied. We noted the blockade of dectin-1 by glucan-P inhibited cytokine production induced by all C. albicans strains and similarly, MR blockade resulted in decreased IL-1β release after stimulation with all of the strains. However, TNF production was inhibited in three of the 14 yeast strains. In contrast, recognition by TLR4 was a much more variable feature and strain dependent. Blockade of TLR4 inhibited IL-1β production by seven of the 14 strains (Fig. 1 and Table 2), and TNF production by only one of the yeast strains (Fig.1 and Table 3). However, Bartonella LPS inhibited by more than 90% of the TNF stimulation induced by ultrapure E. coli LPS (4.4 ± 0.8 ng/ml vs. 0.3 ± 0.2 ng/ml, P < 0.05).

Table 2  IL-1β production induced in human PBMC by the various Candida albicans strains, and the effects of the blockade of TLR4, dectin-1 or MR (given as percentage inhibition of control stimulation with the respective strain). TLR4 was blocked by the inhibitor Bartonella quintana LPS (20 μg/ml), dectin-1 by the inhibitor glucan-phosphate (10 μg/ml), and MR by mannan (200 μg/ml).

<table>
<thead>
<tr>
<th>C. albicans</th>
<th>IL-1β (ng/ml)</th>
<th>TLR4 blockade (% inhibition)</th>
<th>Dectin-1 blockade (% inhibition)</th>
<th>MR blockade (% inhibition)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SCS B38619</td>
<td>1.6 ± 0.4</td>
<td>23*</td>
<td>45*</td>
<td>42*</td>
</tr>
<tr>
<td>SCS 154044</td>
<td>1.8 ± 0.5</td>
<td>-7</td>
<td>35*</td>
<td>38*</td>
</tr>
<tr>
<td>SCS 503505</td>
<td>1.4 ± 0.4</td>
<td>28*</td>
<td>42*</td>
<td>77*</td>
</tr>
<tr>
<td>SC 5314</td>
<td>0.9 ± 0.3</td>
<td>8</td>
<td>38*</td>
<td>67*</td>
</tr>
<tr>
<td>AM 2005/0372</td>
<td>2.6 ± 0.9</td>
<td>19*</td>
<td>62*</td>
<td>38*</td>
</tr>
<tr>
<td>AM 2005/0387</td>
<td>3.8 ± 0.8</td>
<td>5</td>
<td>27*</td>
<td>41*</td>
</tr>
<tr>
<td>AM 2005/0370</td>
<td>1.2 ± 0.4</td>
<td>11</td>
<td>33*</td>
<td>33*</td>
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<tr>
<td>ATCC 18804</td>
<td>0.8 ± 0.3</td>
<td>32*</td>
<td>48*</td>
<td>72*</td>
</tr>
<tr>
<td>J 990102</td>
<td>1.7 ± 0.5</td>
<td>41*</td>
<td>62*</td>
<td>38*</td>
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<tr>
<td>JIMS 103102</td>
<td>1.9 ± 0.6</td>
<td>27*</td>
<td>34*</td>
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<tr>
<td>MYA 3573</td>
<td>2.9 ± 1.1</td>
<td>36*</td>
<td>28*</td>
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<td>78/028</td>
<td>2.4 ± 0.7</td>
<td>5</td>
<td>7</td>
<td>23*</td>
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<tr>
<td>81/078</td>
<td>2.7 ± 0.7</td>
<td>4</td>
<td>22*</td>
<td>45*</td>
</tr>
<tr>
<td>RV 4688</td>
<td>1.2 ± 0.5</td>
<td>-5</td>
<td>41*</td>
<td>74*</td>
</tr>
</tbody>
</table>

Control stimulation are given as means ± SEM. *P < 0.05.
**Disseminated C. albicans infection in TLR4-/- mice**

The experiments performed *in vitro* identified *C. albicans* strains for which TLR4 recognition is required, and strains for which TLR4 seems to play at most a redundant role for recognition. In order to assess whether these differences have consequences *in vivo*, we infected TLR4-/- mice with either a strain for which TLR4 seems to play an important role (SCS 503505) and a strain for which TLR4 does not seem to be important (AM 2005/0370). As depicted in Fig. 2, TLR4-/- mice displayed an increased fungal outgrowth in the kidneys after infection with SCS 503505 strain (panel A), but not after infection with the AM 2006/0370 strain (panel B).

**Discussion**

Using a panel of 14 different *C. albicans* strains, we demonstrate here that recognition of *C. albicans* by lectin receptors is a characteristic that is independent of the strain studied, whereas recognition by TLR4 is highly variable and dependent on the *Candida* strain.

The observation that the recognition by lectin receptors is independent of the strain strongly suggests that the ligands for these receptors are invariably expressed. This apparently holds for the β-glucans, which are crucial *Candida* cell wall components that are being recognized by dectin-1 [14], as well as for the main branched N-linked mannan component recognized by the mannose receptor [10]. β-glucans represents a crucial component responsible for the architecture of the cell wall that is exposed on the surface at the site of budding scars, and its recognition by dectin-1 is crucial for proper innate immune system activation [15,16].

It must be assumed likewise that the differences observed in TLR-4 recognition are due to variation in the expression on the *Candida* cell surface of the components specifically recognized by this pattern recognition receptor. TLR4 recognizes the short linear O-linked mannan structures [10], and the limited availability of these mannans for recognition on the surface of certain *Candida* strains may be due to either differential expression, or to steric hindrance related to the more abundant N-linked mannans. An important point that needs to be mentioned is the assessment of...
heat-killed yeasts in our experiments. The decision to use heat-killed yeasts for the in vitro stimulation experiments was based on previous studies that demonstrated that no major differences can be seen between the role of TLR4 for the recognition of mannans from live or heat-killed Candida [10]. In order to avoid differences in cytokine induction due to variations in growth in the stimulation wells, we chose to use heat-killed yeasts in the stimulation experiments. However, one cannot completely exclude differences in recognition of heat-killed vs. live yeasts of some of the Candida strains used in the experiments. The reason to use the yeast-phase for the stimulation experiments was based on previous studies that have shown that only Candida strains, and not the hyphae, stimulate cytokine production through TLR4 [25].

Based on these findings, we asked whether differences in TLR4 recognition in vitro would have implications for the course of invasive Candida infection in vivo. We found that TLR4-/- mice are more susceptible only to infections induced by a strain for which TLR4 recognition has been shown to play a role in our in vitro experiments, but not to infection due to a strain that was not recognized by TLR4 in vitro. Although the in vivo experiments focused solely on two of the Candida strains tested in vitro, the strong correlation between the in vitro and in vivo results provides the proof-of-principle that differences exist in the recognition of various Candida strains by TLR4. This finding provides an explanation for the variable results that have been reported on pattern recognition of Candida and host defense by various groups.

An indirect consequence of our findings, is that recognition of Candida by lectin receptors is a much more consistent feature than the recognition, and likely more important for antifungal defense. This hypothesis is supported by recent clinical observations that patients with a defect in MyD88 (and consequently in TLR4-mediated pathways) suffer from bacterial, but not from fungal infections [29]. In contrast, patients with defects in Dectin-1 or its adaptor molecule CARD-9 display an increased susceptibility to fungal infections [30,31]. Our own small association study that suggested a role for the Asp299Gly/Thr399Ile TLR4 polymorphisms for susceptibility to disseminated candidiasis [32], does not seem to be corroborated in larger cohorts (Plantinga, manuscript in preparation).

In conclusion, TLR4 is important for the recognition of some, but not all, strains of Candida. In contrast, recognition of cell wall polysaccharides by the lectin receptors Dectin-1 and MR is a constant characteristic of Candida recognition by the innate immune system, and these findings suggest a heavier reliance of in vivo antifungal host defense on lectin receptors than on TLRs, a notion compatible with the clinical picture in individuals deficient in MyD88/TLRs vs. Dectin-1/CARD9.

Acknowledgements

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Declaration of interest: The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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Pattern recognition of Candida albicans


Van der Graaf CA, Netea MG, Morre SA, *et al.* Toll-like receptor 4 Asp299Gly/Thr399Ile polymorphisms are a risk factor for *Candida* bloodstream infection. *Eur Cytokine Netw* 2006; 17: 29–34.

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