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Immunological Features of Macrophages Induced by Various Morphological Structures of *Candida albicans*

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Candida albicans is a dimorphic fungus that commensally colonizes human mucosal surfaces. The aim of this study was to assess the role of different *C. albicans* morphologies in inducing pattern recognition receptors (PRRs) and cytokines in macrophages. Macrophages may respond to pathogen-associated molecular patterns *via* TLR2 and TLR4 by expressing cytokines. The hyphal transition of *C. albicans* was induced by 20% serum (S), RPMI-1640 (R), or 39°C culture (H). Macrophages were then challenged with either yeast (Y) or different hyphae cultures of *C. albicans*, followed by RT-PCR and FACS analysis of PRRs expression. In addition, macrophages were stimulated with either yeast or different hyphae cultures of *C. albicans* used by RT-PCR and Bio-Plex analysis of cytokines production. Macrophages expressed high levels of TLR4 and dectin-1 after stimulation with Y cells. In contrast, stimulation with H or R cells strongly increased the expression of TLR2 and dectin-2. Stimulation with Y cells significantly enhanced the expression of IL-1β and weakly increased the expression of IL-6 and IL-12. Stimulation with hyphal cells (S, R, and H) strongly increased IL-10 expression, but weakly reduced IL-1β expression. The phagocytosis activity and NO production of macrophages were decreased upon treatment with hyphal cells compared with yeast, and depended on the length of hyphae. In summary, the yeast and hyphae forms of *C. albicans* resulted in an induction of different PRRs, with accompanying differences in immune cell cytokine profiles.

Keywords: *Candida albicans*, TLR, dectin, interleukins

Introduction

Candida albicans is usually not harmful, but it can cause severe disease in immune-deficient patients. The innate immune system acts first to remove *C. albicans* [12, 14]. This system recognizes a wide range of pathogens by utilizing the limited repertoire of germline-encoded proteins such as pattern recognition receptors (PRRs). Human PRRs recognize specific pathogen-associated molecular patterns (PAMPs) that are not present in humans but are commonly expressed by pathogens. PRRs that recognize PAMP include Toll-like receptors (TLRs), NALPs, dectins, retinoic acid-inducible gene I, melanoma differentiation-associated antigen 5, and DNA-dependent activator of interferon regulatory factor. TLRs recognize *C. albicans* [2, 3, 5, 16] and comprise a new group of cellular receptors that mediate inflammatory reactions against microorganisms [8].

Studies conducted in mice have revealed that several types of TLRs are expressed, and they induce the innate immune response by recognizing not only virus but also bacteria, fungi, and protozoa [13]. So far, 10 types of human TLRs and 13 types of mice TLRs have been discovered. Although most TLRs contain similar leucine-rich repeats as an extracellular domain, each TLR recognizes different PAMPs through the extracellular domain. Each TLR recognizes several types of PAMP. Thus, a limited number of TLRs can potentially react with diverse microorganism products [1, 4, 9, 17, 22].

The cell wall of *C. albicans* is composed of chitin, glucan, mannan, mannoprotein, and glycolipid that are completely

different from the components of the bacterial cell wall or viral envelop, and the composition varies according to the morphology of *C. albicans* [11, 21]. Among the components, mannan is known to react with both TLR4 and CD14, phospholipomannan is recognized by TLR2 leading to the production of pro-inflammatory cytokines, and β-glucan is recognized by immune cells through dectin-1 [10, 19]. Innate immunity is the first line of defense against *C. albicans* infection, and appropriate inflammatory reactions and consequent cytokine secretion by immune cells play important roles. Unlike other fungi, *C. albicans* changes its morphology according to the external environment and morphologically presents as the yeast or the hyphae. Host cells recognize such morphological changes of *C. albicans* differently, thus stimulating different immune responses. In this study, we first examined the expression pattern of TLRs in macrophages according to the morphology of *C. albicans* to assess the control of immune responses against *C. albicans* mediated by TLRs. We then investigated the pattern of cytokine expression to assess the difference in inflammatory reactions induced by *C. albicans* with either yeast or hyphae morphology.

Materials and Methods

Animals

Balb/c mice were obtained from Orient Bio (Republic of Korea). Animals were used at 8−12 weeks of age. Animals were kept and handled in accordance with institutional guidelines.

Candida albicans

C. albicans ATCC 10231 was obtained from Korea Centers for Disease and Prevention and was used in all experiments. The yeast cells were kept on Sabouraud dextrose agar (SDA; Difco, Becton Dickinson, USA), at 30°C, and were grown in Yeast extract Peptone Dextrose (YPD) broth for 24 h at 30°C. The yeast cells were then harvested by centrifugation. To generate hyphae, live yeast form of *C. albicans* were grown for 6 h, (i) at 39°C in YPD medium, (ii) at 37°C in RPMI-1640 (Gibco-BRL, USA), and (iii) at 37°C in YPD medium supplemented with 20% heat-inactivated fetal bovine serum (FBS). After 6 h, more than 90% of blastoconidia were grown to hyphae, as confirmed by microscopes. The yeast and the hyphae were heat killed for 1 h at 56°C.

Isolation of murine peritoneal macrophages

Isolation of macrophages was performed as described previously [23]. Briefly, mice were injected intraperitoneally with 1 ml of thioglycollate (3% (w/v); Becton Dickinson, USA), and the peritoneal cells were harvested after 72 h by washing the cavities with 2% FBS-phosphate buffer saline (PBS). Macrophages were centrifuged (5 min; 2,000 rpm), counted in a hemocytometer, and resuspended in RPMI-1640 supplemented with 10% FBS (Hyclone, USA) and 1% penicillin/streptomycin (Gibco-BRL). Cells were washed once with PBS and plated at a density of 5×10^6 cells in 3 ml medium per well in a 6-well tissue culture plate. Peritoneal macrophages were allowed to adhere for 2 h at 37°C in a 5% CO_2 atmosphere and the non-adherent cells were removed.

Cytokine Expression on Peritoneal Macrophages

Peritoneal macrophages were stimulated with *C. albicans* yeast form, various hyphae forms (5:1 *Candida*:macrophage ratio based on optimal stimulation), or lipopolysccharide (LPS, 100 ng/ml; Sigma, USA) for 6 h in 5% CO_2 at 37°C. Total RNAs were extracted using TRIzol (Invitrogen, USA) according to the manufacturer's instructions. First-strand cDNA synthesis was performed using the Superscript II RT kit with an oligo(dT) primer (Invitrogen), as described by the manufacturer. PCR amplification was carried out with the following primer sets: 5′-GTGGTACCTGAGAATGAT GTGGG-3′ (forward), 5′-GTTAATTAAGTCAGGAACTGGGTG-3′ (reverse) for TLR2; 5′-CTGGGTGAGAAATGAGCTGG-3′ (forward), 5′-GATACAATTCCACCTGCTGCC-3′ (reverse) for TLR4; 5′-GAA CCACAAGCCCACAGAAT-3′ (forward), 5′-CATGGCCCTTCA CTCTGATT-3′ (reverse) for dectin-1; 5′-GCTAGCTGCTGTGAT TTCCA-3′ (forward), 5′-TGAAACACACCGCTCTTCTG-3′ (reverse) for dectin-2; 5′-TACAGGCTCCGAGATGAACAACAA-3′ (forward), 5′-TGGGGAAGGCATTAGAAACAGTC-3′ (reverse) for IL-1β; 5′-TTGTGGTCTCTCTTGCTGGT-3′ (forward), 5′-TGAAGCCTCCCT AATAGACC-3′ (reverse) for IL-6; 5′-CTTCTTCAGCAACAGCAA GG-3′ (forward), 5′-TGAGCTCATTGAATGCTTGG-3′ (reverse) for IFN-γ; 5′-CACCTGCCCAACTGCCGAGG-3′ (forward), 5′-GCACGCAGACATTCCCGCCT-3′ (reverse) for IL-12; 5′-ATG CAGGACTTTAAGGGTTACTTGGGT-3′ (forward), 5′-ATTTCG GAGAGAGGTACAAACGAGGTTT-3′ (reverse) for IL-10; and 5′-GTGGGCCGCTCTAGGCACCAA-3′ (forward), 5′-CTCTTTGAT GTCACGCACGATTTC-3′ (reverse) for β-actin. β-Actin was used as an internal control.

Flow Cytometery

For detection of surface molecules, cells were resuspended at 1×10^7 cells/ml in reaction buffer (1% BSA-PBS) and incubated with 1 μg of anti-mouse CD16/CD32 for 20 min on ice prior to staining. An aliquot (100 μl) of cell suspension was added to 96-well plates containing a mixture of monoclonal antibodies: anti-mouse TLR2 FITC, anti-mouse TLR4, PE and anti-Dectin-1 PerCP (eBioscience Inc., USA); anti-mouse Dectin-2α APC (R&D Systems, USA). The cells were incubated for 1 h on ice, and then washed twice with reaction buffer. The samples were analyzed on a FACS ariaIII (BD Biosciences, USA) flow cytometer using the FCS express software.

ELISA

The levels of cytokines (IL-1β, IL-6, IL-10, IL-12p70, and IFN-γ) in the culture supernatants were measured using the Bio-Plex multiplex cytokine assay system (Bio-Rad, USA). Net cytokine

levels indicate cytokine values following subtraction of baseline values. Cytokine-specific antibody-coated beads (Bio-Rad) were used for these experiments. The assay was performed according to the manufacturer's instructions. Cytokine concentrations were automatically calculated with the Bio-Plex Manager software by using a standard curve derived from a recombinant cytokine standard.

Analysis of Phagocytosis by Macrophages

To quantitate the internalized fungi, peritoneal macrophages (2×10^5) were incubated at 37°C with *C. albicans* yeast or hyphae (5:1 *C. albicans*:macrophages ratio) for 1 and 3 h. Cells were then washed twice with PBS to remove excess unbound cells. Macrophages were lysed in ice-cold distilled water for 20 min. The cell lysate was diluted with PBS and inoculated on SDA plates. The colony forming unit (CFU) number was counted 24 h after incubation.

Nitric Oxide Production

For the determination of *in vitro* NO production, the production of nitrite (NO_2-) was measured in the supernatants of cultivated macrophages. Macrophages (5×10^5 cells) were incubated with medium, the yeast or the hyphae forms of *C. albicans*, or 100 ng/ml of LPS (Sigma) for 24 h at 37°C and 5% CO_2. The supernatant was collected and the total amount of NO_2- was determined by the Griess method [7]. Briefly, 100 µl of supernatant samples was incubated with an equal volume of Griess reagent at room temperature for 15 min. The absorbance was measured on a microtiter plate reader (Emax; Molecular Devices, USA) at 540 nm. The NO_2- concentration was determined using a $NaNO_2$ standard curve at a concentration range of 1–80 µM.

Statistical Analysis

Statistical analysis was performed using a Student's *t* test. Triplicate experimental samples were compared with control samples. The level of significance between groups was set at $p < 0.05$. Data are given as the mean ± SEM.

Results

PRRs of Macrophages That Recognize *C. albicans*

Several factors that induce the switch of *C. albicans* morphology from yeast to hyphae were examined. First, *C. albicans* was inoculated and grown in YPD medium at 39°C. Second, it was cultured in RPMI-1640 (pH 6.4). Third, it was inoculated into YPD medium containing 20% serum and cultured at 37°C. The hyphae length was estimated by a mean of 100 hyphae under a microscope. The hyphae of *C. albicans* cultured at high temperatures had pseudohyphae morphology with short hyphae lengths. The length of the high temperature-induced hyphae form was 12 ± 3 µm. Hyphae formed under nutritional deprivation were the longest, where the length of the hyphae form was 55 ± 10 µm, and aggregated over time. Long hyphae were also formed in cultures grown in YPD medium containing 20% serum, where the length of hyphae was 41 ± 7 µm (Fig. 1A).

The cell wall of *C. albicans* forms a network of glucan, chitin, and other polysaccharides, and contains mannosylated proteins. Murine macrophages were treated with either the yeast or the hyphae forms of *C. albicans* that had been induced by various conditions, and the expressions of cellular PRRs were compared. TLR2 mRNA expression, which is known to recognize whole cell of *C. albicans*, was higher on macrophages treated with the hyphae compared with the yeast form of *C. albicans*, and the highest for the hyphae formed during nutritional deprivation. TLR4 mRNA expression, which recognizes α-mannan of *C. albicans*, was high in macrophages treated with the yeast form of *C. albicans* or with LPS. TLR4 mRNA expression was not increased in macrophages treated with the hyphae form of *C. albicans*. The dectin-1 receptor of macrophages is known to recognize β-glucan of *C. albicans*. We found that the mRNA expression level of dectin-1 increased on macrophages treated with the yeast form of *C. albicans*, but not on macrophages treated with the hyphae form. The component of *C. albicans* cell wall that binds to dectin-2 is unknown. Dectin-2 is known to recognize other components, and dectin-2 mRNA expression did not increase in macrophages treated with LPS or the yeast form of *C. albicans*. Dectin-2 mRNA expression was increased in macrophages treated with the hyphae form of *C. albicans*, particularly when the hyphae were induced by nutrition deprivation (Figs. 1B, 1C). Moreover, we determined the surface expression of the PRR by flow cytometry in macrophages treated with the yeast form or the hyphae form of *C. albicans*. The results were shown to be a similar pattern in the expression of the PRR mRNA. TLR2 was more highly expressed in the macrophages stimulated with the hyphae form than the yeast form. As for treatment of the yeast form, TLR4 expression was markedly increased, but it was not expressed as treatment of the hyphae form in macrophages. When the yeast form or the high temperature-induced hyphae form were treated, dectin-1 expression was enhanced. However, dectin-1 did not express in macrophages treated with the other hyphae form. Only dectin-2 expression was significantly increased in the hyphae form-treated macrophages (Fig. 2).

Our findings suggested that macrophages express different PAMPs depending on the morphology of yeast or differences in the composition of the *C. albicans* cell wall, which may influence the activation of a series of immune responses induced by binding to different PRRs.

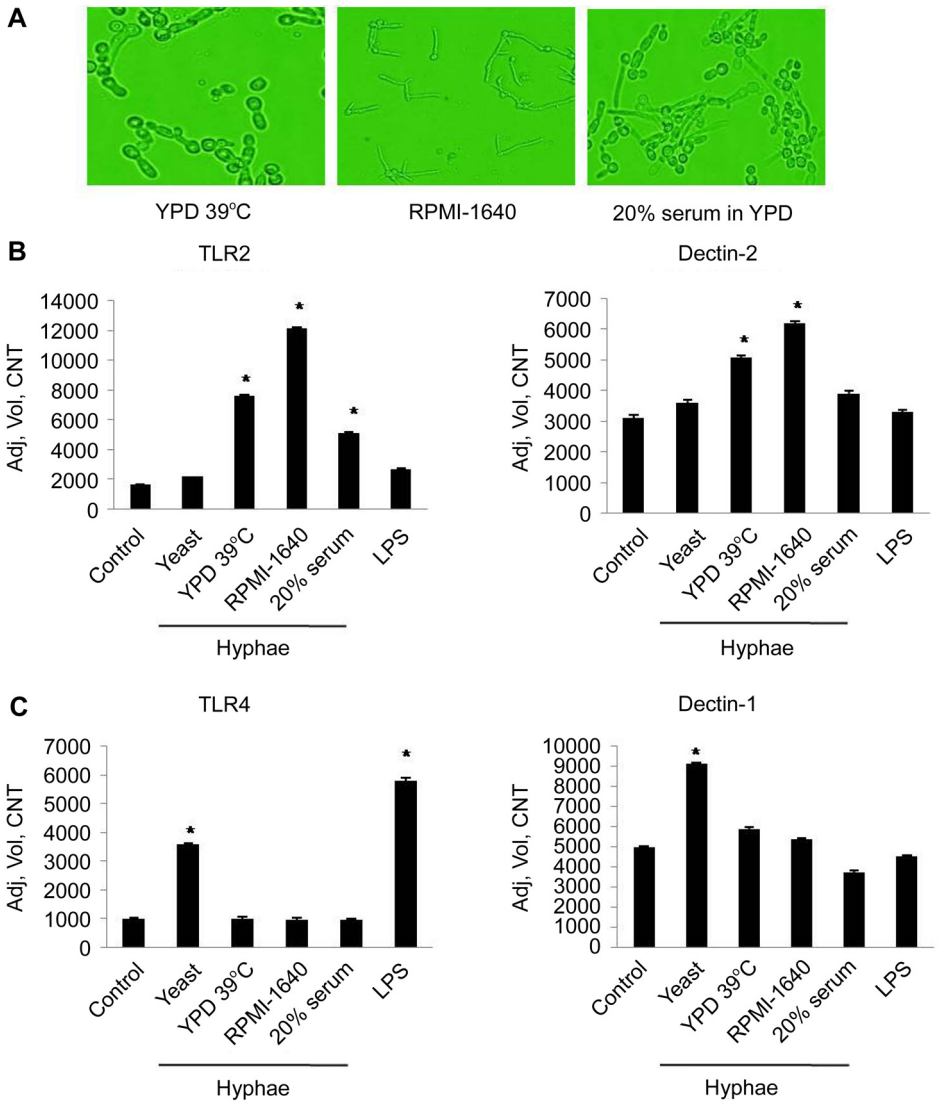

Fig. 1. *C. albicans* morphology, and its effect on PRRs expression in macrophages.

(**A**) Morphology of *C. albicans* under different culture conditions. *C. albicans* was grown for 6 h at 39°C in YPD, or at 37°C in RPMI-1640, or in YPD supplied with 20% serum. Coverslips were mounted on slides and viewed by light microscopy. PRR expression in macrophages upon challenge by yeast or hyphal cells was investigated. Expressions of TLR2, and dectin-2 (**B**), and TLR4, and dectin-1 (**C**) in macrophages. Mice peritoneal macrophages (5×10^6) were co-cultured with heat-inactivated *C. albicans* yeast or hyphal cells at a *C. albicans*/macrophage ratio of 2:1 for 6 h. Total RNAs were extracted and mRNA expression levels were examined by semiquantitative RT-PCR. Data shown are the mean value of results from three independent experiments. Significant values are given as $p < 0.05$ (*).

Effect of *C. albicans* with Yeast or Hyphae Morphology on the Expression of Cytokines in Murine Macrophages

When macrophages recognize sources of infection through receptors, the secretion of several types of cytokines is stimulated, which activates macrophages themselves by autocrine effects and stimulates other immune cells by paracrine effects. Murine macrophages were treated with *C. albicans* having a yeast or hyphae morphology induced under several conditions, and cytokine expression was assessed. When microorganisms invade the human body, inflammation is induced to remove them. The mRNA of IL-1β, a pro-inflammatory cytokine, was up-regulated in macrophages treated with LPS. The IL-1β mRNA expression was elevated when macrophages were treated with the yeast form of *C. albicans*, but the hyphae form of *C. albicans* did not have this effect. The IL-6 mRNA expression was also elevated when macrophages were treated with the yeast form of *C. albicans*. Comparable elevated IL-6 mRNA

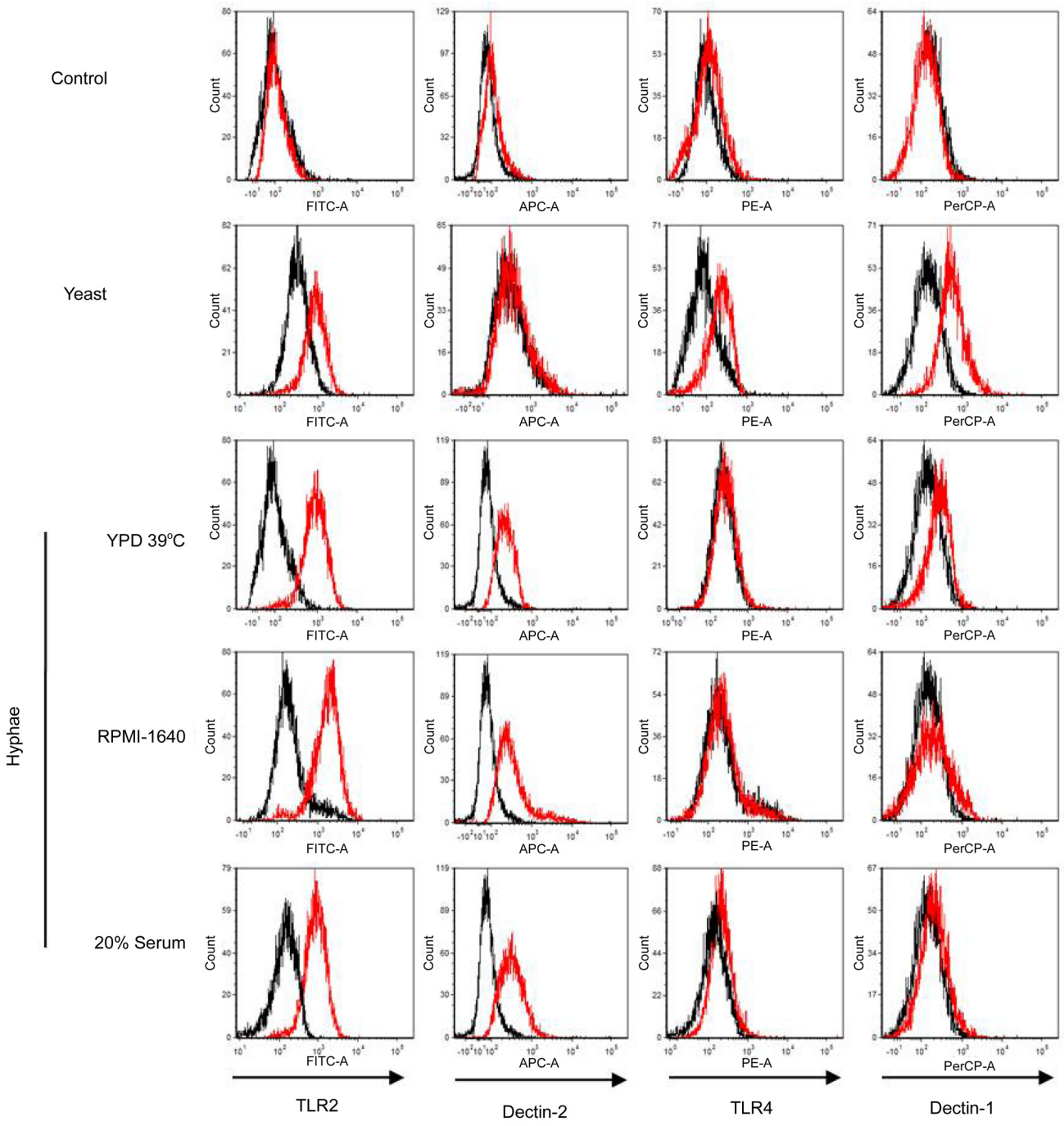

Fig. 2. TLR2, dectin-2, TLR4 and dectin-1 expressions on peritoneal macrophages.
The cells were stimulated with heat-inactivated *C. albicans* yeast or hyphae cells for 18 h. The peritoneal macrophages were stained with anti-mouse TLR2 FITC, anti-mouse TLR4 PE, anti-dectin-1 PerCP, anti-mouse dectin-2α APC (red line), or matched isotype control antibodies (black line) and analyzed by FACS.

expression occurred when macrophages were treated with *C. albicans* having a hyphae morphology induced at high temperatures. However, the other hyphae that were induced by RPMI-1640 and 20% serum did not increase IL-6 mRNA expression (Fig. 3A). IFN-γ is secreted by macrophages and plays an important role in phagocytosis

by inducing their activation. The IFN-γ mRNA expression was elevated in macrophages treated with *C. albicans* in the yeast form and high temperature-induced hyphae form. When macrophages were treated with other hyphae, the IFN-γ mRNA expression decreased. Thus, the phagocytic activity of macrophages treated with *C. albicans* in the hyphae morphology was suppressed, and microorganisms could therefore evade this host immune response. IL-12 plays an important role in the differentiation of T cells. The IL-12 mRNA expression was also elevated above controls when macrophages were treated with the yeast form of *C. albicans*, but not by the hyphae form. The elevated IL-12

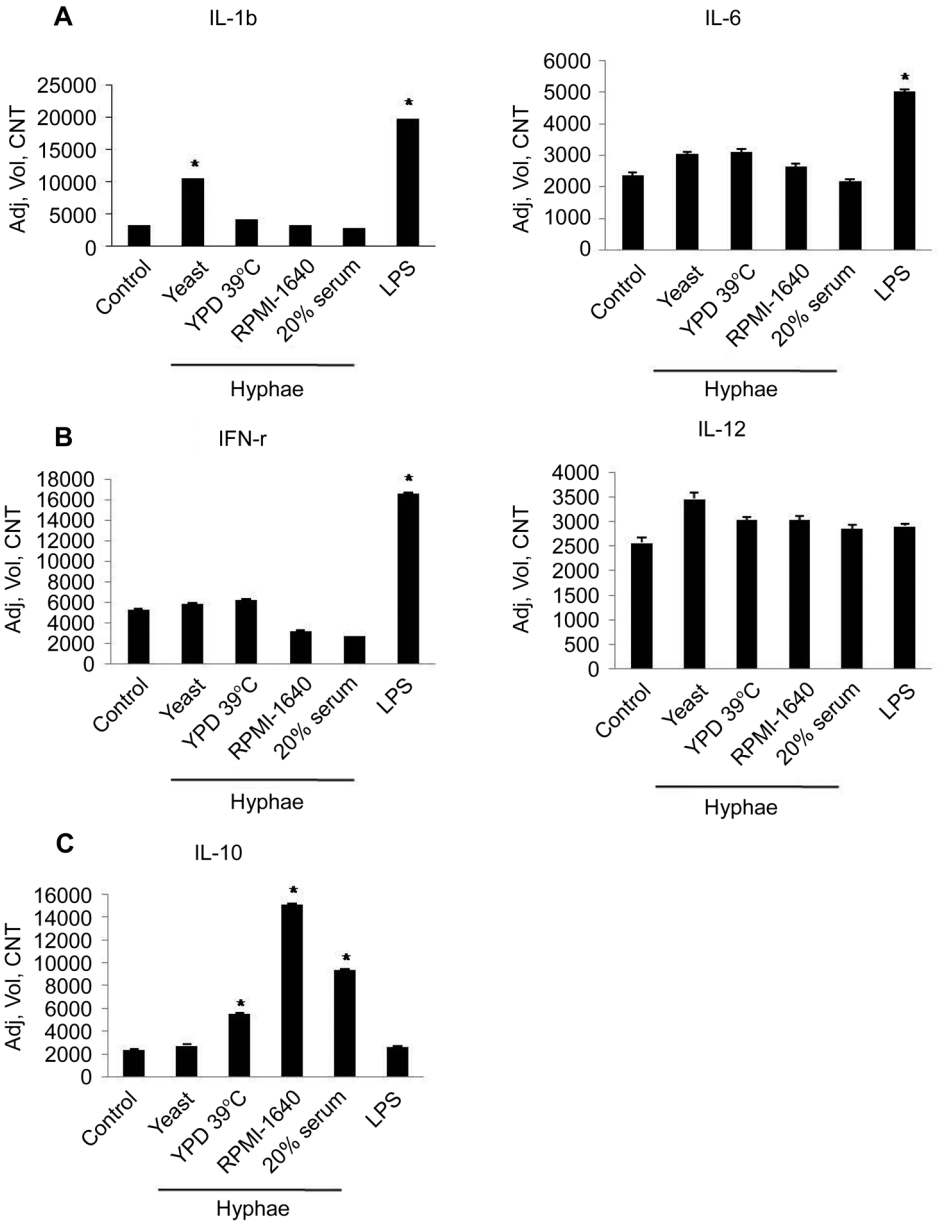

Fig. 3. Effect of *C. albicans* dimorphism on cytokine expression in mouse peritoneal macrophages.
(**A**) Inflammatory cytokine, IL-1β and IL-6 expression, (**B**) Th1 response cytokine, IFN-γ and IL-12 expression, (**C**) Anti-inflammatory cytokine, IL-10 expression, by macrophages in response to *C. albicans*. Mice peritoneal macrophages (5×10^6) were co-cultured with heat-inactivated *C. albicans* yeast or hyphal cells at a *C. albicans*/macrophage ratio of 2:1 for 6 h. Total RNAs were extracted and mRNA expression levels were examined by semiquantitative RT-PCR. Data shown are the mean value of results from three independent experiments. Significant values are given as $p < 0.05$ (*).

mRNA expression by *C. albicans* with the yeast morphology would lead to the induction of T cell differentiation to Th 1 type cells (Fig. 3B).

Induction of appropriate inflammatory reactions is essential for the removal of invading microorganisms. Nonetheless, excessive inflammation can induce serious injury to hosts, and thus it is important to properly control inflammatory reactions. When macrophages were treated with *C. albicans* in the yeast morphology, the level of mRNA expression of IL-10, an anti-inflammatory cytokine, was comparable to the control group. However, *C. albicans* in the hyphae morphology led to a rapid elevation of IL-10 mRNA expression (Fig. 3C).

Additionally, we assessed the protein production of cytokines in the supernatant, confirmed by Bio-Plex assay. Introduction of the yeast form or LPS to macrophages led to significantly enhanced amount of IL-1β and IL-6, but the hyphae form did not. The IL-12p70 production was markedly increased in macrophages treated with the yeast form. IL-10 production was enhanced in macrophages treated with the hyphae form (Fig. 4). INF-γ, however, was not detected at all (data not shown).

Effect of *C. albicans* in Yeast or Hyphae Morphology on the Phagocytic Activity of Macrophages

Macrophages were infected with live *C. albicans* with the yeast or hyphae morphology, and the number of *C. albicans* phagocytized within them was examined 1 and 3 h later. When macrophages were infected with *C. albicans* in the yeast morphology, 1.5×10^4 *C. albicans* were phagocytized in 1 h. For *C. albicans* in the hyphae morphology induced at 39°C, 1.32×10^4 cells were phagocytized, which was less than

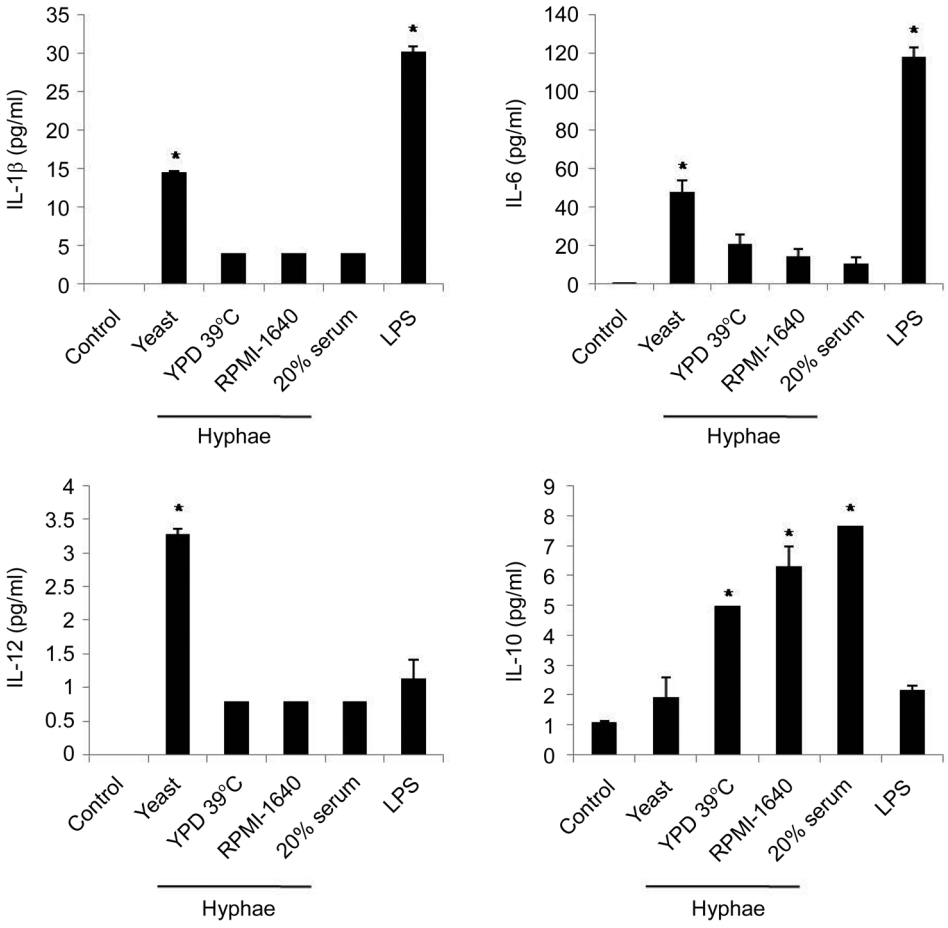

Fig. 4. Effect of *C. albicans* dimorphism on cytokine production in mouse peritoneal macrophages.
Production of IL-1β, IL-6, IL-12p70, and IL-10 by macrophages after stimulation with *C. albicans* yeast or hyphal cells or LPS (10 μg/ml). The cells were stimulated in triplicates for 48 h. The cytokine production in the supernatant was measured in the Bio-Plex assay. Data are expressed as the mean ± SEM ($N = 4$) of separate experiments. Significant values are given as $p < 0.05$ (*).

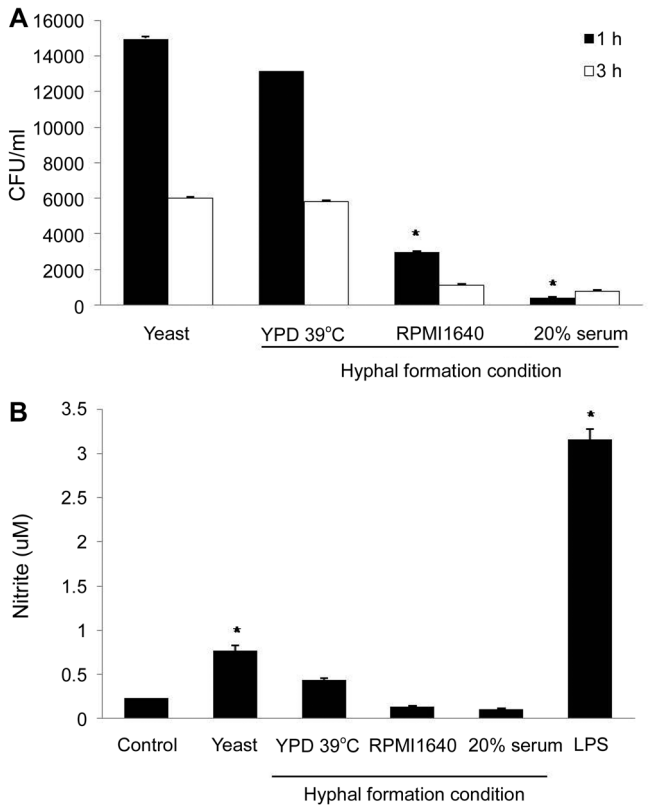

Fig. 5. Effect of *C. albicans* dimorphism on macrophage phagocytic activity.

(**A**) Mice peritoneal macrophages (2×10^5) were co-cultured with live *C. albicans* (1×10^6) yeast or hyphal cells for 1 or 3 h. Phagocytic macrophages were destroyed with water and spread on a SDA plate. (**B**) NO production in peritoneal macrophages in the presence of heat-inactivated *C. albicans* yeast or hyphal cells. Peritoneal macrophages (5×10^5 cells/well) were stimulated with the indicated condition of heat-inactivated *C. albicans* (1×10^7 cells/well) or LPS (1 μg/ml) for 24 h and levels of nitrite were determined. Data are expressed as the mean ± SEM ($N = 9$) of separate experiments. Significant values are given as $p < 0.05$ (*).

that obtained for the yeast type. When hyphae had been induced by nutrition depravation, 3×10^3 were phagocytized. In macrophages infected with serum-induced hyphae-type *C. albicans*, 4.2×10^2 were phagozytized and phagocytosis was rapidly reduced. When macrophages were reacted with *C. albicans* for 3 h, the number of detected phagocytized *C. albicans* was reduced. This did not reflect a reduction in phagocytosis, but was rather due to lysis of *C. albicans* within macrophages. The reason underlying the differences in the number of *C. albicans* phagocytized by macrophages depending on their morphology might be that several *C. albicans* in the yeast morphology were phagocytized in one macrophage, but the length of hyphae of *C. albicans* in the hyphae morphology was too long, and thus macrophages were unable to phagocytize them properly (Fig. 5A).

A substance that is closely involved in the host defense mechanism for the removal of pathogens is NO. To examine whether the formation of NO that mediates effects on macrophage phagocytosis was influenced by the morphology of *C. albicans*, macrophages were treated with different forms of *C. albicans*, and NO was measured 24 h later. NO was increased 2.5-fold compared with the control, by the yeast form. In contrast, NO rapidly decreased upon treatment with the hyphae form. We therefore concluded that when macrophages were infected with the hyphae form, phagocytosis decreased owing to impaired formation of NO, despite the increased length of hyphae (Fig. 5B).

Discussion

C. albicans is an opportunistic strain that is present in most human skin. In individuals with compromised immunity, it invades the host systemically and induces diseases. Both innate and adaptive immunity are involved in the removal of *C. albicans* from the human body. The defense is thus mediated by polymorphonuclear leukocytes, complement, antibody, and mononuclear cellular responses. *C. albicans* may change from having a yeast morphology to a hyphae morphology as a result of genetic or environmental factors [4, 9]. The effect of this dimorphism on virulence has not been elucidated.

In this study, we investigated whether yeast-type and hyphae-type *C. albicans* induced by various conditions resulted in a different stimulation of macrophages. We examined both mRNA and protein expression of PRRs. Yeast-form *C. albicans* elevated the expression of the cell surface receptors TLR2 and TLR4 in comparison with the control group, and the expression of TLR4 was elevated rapidly. On the other hand, when macrophages were treated with *C. albicans* in hyphae morphology, the expression of TLR4 was not elevated. This implies that the hyphae are recognized by TLR2, but not TLR4. The reactions of the C-type lectin-like receptors dectin-1 and dectin-2 were also examined. The expression of dectin-1 that recognizes β-glucan was elevated when macrophages were treated with *C. albicans* in the yeast, but not in the hyphae, morphology. Expression of dectin-1 is known to be associated with macrophage phagocytosis [5, 20], and if the expression of dectin-1 is decreased, phagocytosis of the fungus would be compromised. β-Glucan that is recognized by dectin-1 is distributed abundantly in the bud scar area of the yeast [5,

6]. When hyphae are formed, they cover bud scars and thus induce a weak innate immune response. Elevation of the expression of dectin-2 was confirmed to be specific to hyphae. In conclusion, yeast- and hyphae-types *C. albicans* induced the expression of different receptors on macrophages. This is considered to be due to the different composition of the cell walls of the two morphological types of *C. albicans* [20].

We also assessed the mRNA expression and protein production of cytokines as a host defense mechanism by different morphological forms of *C. albicans*. As the morphology of *C. albicans* was switched from yeast to hyphae, *C. albicans* that previously reacted with TLR4 switched to TLR2, the expression of IFN-γ was decreased, the expression of pro-inflammatory cytokines was decreased, and the expression of IL-10 was elevated. It appears that the elevation of expression of IL-10 allowed *C. albicans* hyphae to evade the immune response of host cells. Chitin is a component of the inner wall and plays an important role in the shape and viability of cells [15]. Chitin is present in *C. albicans* in all morphologies; nonetheless, it has been reported to be present more in the hyphae than in the yeast form [20]. Upon heating, chitin is expressed in the outer wall. The receptor to which this chitin binds has not been elucidated. Nevertheless, it is known to react with macrophages and to suppress the expression of inflammatory cytokines. One reason for the reduction of expression of inflammatory cytokines induced by the hyphae-form *C. albicans* may be the increase in chitin within the cell wall compared with the yeast form [18].

Finally, we assessed the level of phagocytosis of macrophages induced by different morphologies of *C. albicans*. The phagocytosis of the hyphae form of *C. albicans* by macrophages was rapidly reduced. We found that not only the length of hyphae, but also the decrease in NO synthesis by macrophages were important for the decrease in candidacidal activity and evasion of host immune response by *C. albicans* hyphae.

In summary, *C. albicans* induced different immune responses depending on whether the morphology was yeast- or hyphae-type. First, among different macrophage cell surface receptors, the yeast form of *C. albicans* induced TLR4, and stimulated the synthesis of IL-1β and IL-6 cytokines by binding to dectin-1, and thus induced an inflammatory reaction and the activation of macrophages by increasing IFN-γ synthesis. In addition, by increasing NO synthesis, macrophages phagocytosis was ultimately induced, and *C. albicans* could be eliminated. In contrast, the hyphae form of *C. albicans* bound to TLR2 and dectin-2 and thus decreased the expression of IL-1β and IL-6. Since TLR4 was not bound, IFN-γ synthesis was not induced. Hence, the activation of macrophages was reduced and IL-10 synthesis was increased so that the induction of inflammatory reactions could be blocked. We suggest that because of the decrease of expression of dectin-1 that is closely associated with phagocytosis and the reduction of NO synthesis, the hyphae-type *C. albicans* could resist macrophage phagocytosis and evade the host immune response.

Acknowledgments

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