Abstract: Mannan (mannosylated glycoproteins) in the outermost layer of the *Candida* cell wall may be the first molecules that interact with host dendritic cells (DCs) and activate immune responses that determine disease outcomes. However, little is known about how different mannan structures of common oral *Candida* species affect DC activation. The effects of heat-inactivated (HI) yeast cells and soluble mannan of *Candida albicans*, *Candida parapsilosis*, and *Candida dubliniensis* on bone marrow-derived DC (BMDC) responses were compared. HI *Candida* and the mannan exhibited different effects on BMDC activation and functions, which could be due to other carbohydrate compositions in the yeast cell wall. Among *Candida* mannan, the *C. albicans* mannan was the weakest stimulus and induced only interferon (IFN)-γ production. This suggests the possibility that *C. albicans* mannan may skew T helper (Th) responses from protective Th17 toward Th1. In contrast, *C. parapsilosis* mannan caused strong BMDC activation and high production of several proinflammatory cytokines which possibly promote hyperinflammation.

Meanwhile, *C. dubliniensis* mannan induced moderate BMDC responses, which may correlate with its lower pathogenicity. Therefore, mannan of each *Candida* species play distinct roles in DC responses and may be involved in the immunopathogenesis and disease severity of oral candidiasis as well as other *Candida* infection.

Keywords: mannan; dendritic cells; oral Candidiasis; *Candida albicans*; *Candida dubliniensis*; *Candida parapsilosis*.

Introduction

Oral candidiasis is a common problem in the elderly and in immunocompromised individuals, whose defense mechanisms are impaired locally or systemically. Local and systemic predisposing factors leading to a pathogenic condition include ill-fitting prosthetic appliances, xerostomia, malnutrition, and hormonal and endocrine disorders (1). Although the most common species responsible for oral candidiasis is *C. albicans*, a shift toward non-*albicans* *Candida* species (NACs) has been reported in recent decades because of the widespread use of broad-spectrum antibiotics and prolonged use of antibiotics and antifungal therapy (2,3).

* C. parapsilosis and *C. dubliniensis* are two common NACs isolated as normal flora and from oral candidiasis lesions (4-6). *C. parapsilosis* is a complex comprising *C. parapsilosis*, *C. orthopsilosis*, and *C. metapsilosis*, three
distinct species which are common colonizers of the oral cavity. *C. parapsilosis* has been recognized as a major human pathogen since it has high prevalence worldwide (7). In young patients with cancer, *C. parapsilosis* appears as the second most abundant species, after *C. albicans*, causing oral candidiasis (8). *C. dubliniensis* is closely related to *C. albicans*, and it has been found at high prevalence in the oral cavities of HIV-infected patients and healthy individuals (4,9). In addition, a study on patients with insulin-dependent diabetes mellitus reported that *C. dubliniensis* was the second most common species in the oral environment (10). Additionally, besides oral candidiasis, *C. albicans*, *C. parapsilosis*, and *C. dubliniensis* have recently been isolated from dental caries and periodontitis (11,12).

*Candida* cell wall plays a pivotal role in host-fungal interaction (13). Mannan is a major carbohydrate constituent located in the outermost layer of *Candida* cell wall (13) and may initially activate immune response. Evidence exists that mannans are a significant virulent factor associated with the pathogenesis of *Candida* infection (14,15). Mannans interact with C-type lectin receptors (CLRs) and Toll-like receptors (TLRs) abundantly expressed on antigen presenting cells (APCs), leading to the induction of inflammatory response and host defense mechanisms (13). *C. albicans* mutants with impaired cell wall mannosylation are less virulent and show a reduction in APC activation (16,17).

Dendritic cells (DCs) are potent APCs that can provoke protective immunity against oral candidiasis via the induction of Th cells (17,18). It has been demonstrated that DC activation by *C. albicans* mannan triggered Th17 immunity (16,19). However, the differences in mannan structures could influence DC response (20,21). To date, the effect of NACs mannan on DC immunity is not well understood.

Since there is a diversity in mannan structures among different *Candida* species (22-24), it is hypothesized that DCs may differentially respond to mannan of the yeast form of *C. albicans*, *C. parapsilosis*, and *C. dubliniensis*. Therefore, this study investigated the DC maturation and function in response to immobilized cell wall mannan of HI *Candida* yeasts. In addition, the effect of mannan was evaluated by using soluble mannan extracted from the cell wall of the three *Candida* species.

### Materials and Methods

#### Cultivation of Candida yeasts

Three *Candida* species were selected for our study: *C. albicans* (ATCC 24433), *C. parapsilosis* (ATCC 90018), and *C. dubliniensis* (NCPF 3949 or MYA-646). The strains chosen for *C. albicans* and *C. parapsilosis* are reference strains for quality control and antifungal drug susceptibility testing, whereas the strain for *C. dubliniensis* is the only available standard strain (Microbiologics Inc., St. Cloud, MN, USA). The fungi were grown on YPD agar (2% yeast extract, 1% peptone, 2% dextrose, and 2% agar) (HiMedia, Mumbai, India and Ajax Finechem, NSW, Australia) at 30°C for 2 days. The colonies were subcultured in YPD broth at 30°C with 200 rpm shaking for 8 h to reach the log phase.

#### Preparation of heat-inactivated yeasts

Yeast cells were harvested, washed twice, and diluted to a concentration of 1 × 10⁸ cells/mL in sterile phosphate-buffered saline (PBS) (GIBCO/ThermoFisher Scientific, Rochester, NY, USA). The cells were then inactivated by heating at 100°C for 10 min. The HI yeast cells were collected by centrifugation and resuspended in complete Roswell Park Memorial Institute (RPMI) medium at a concentration of 1 × 10⁷ cells/mL.

#### Isolation and purification of mannan

Mannan extraction was performed as previously described (25), with minor modifications. Briefly, 100 g of wet *Candida* yeast cell pellets were resuspended in 250 mL of citrate buffer at pH 7.0, 0.02 M for *C. albicans*, and 0.1 M for *C. parapsilosis* and *C. dubliniensis*. For the mannan extraction of *C. parapsilosis* and *C. dubliniensis*, 0.1 M citrate buffer was used because the lower concentrations (0.05 M and 0.02 M) could not extract the mannan from these organisms. To rule out the possibility that different buffer concentrations could affect the results, the *C. albicans* mannan that was extracted with 0.1 M and 0.02 M citrate buffer were compared, and no difference was found in the amount of mannan or bone marrow-derived dendritic cell (BMDC) responses (data not shown). Next, the yeast suspension was autoclaved at 121°C for 90 min, and the supernatant was separated and collected. The remaining yeast cells were resuspended in 375 mL of the same buffer and re-autoclaved at 121°C for 90 min. The supernatant from the second round of autoclaving was collected and combined with that from the first round. Equal volume of Fehling’s solution was then added to the supernatant, and the mixture was stirred overnight at 4°C. The sediment was collected and dissolved in 6-8 mL of 3 N hydrochloric acid (HCl), and the solution was added drop-wise into 100 mL of methanol-acetic acid (ratio 8:1, v/v). The mannan precipitate was collected by centrifugation and washed with HCl and methanol-acetic acid repeatedly until the precipitate becomes colorless. Mannan was then dissolved in sterile water. *C. albicans*
mannan was further dialyzed in sterile water. Since the dialysis method caused a tremendous loss of C. parapsilosis and C. dubliniensis mannan, Biogel P-4 (Bio-Rad, CA, USA) column was used as the alternative method for changing the buffer. The concentration of mannan was determined using the phenol-sulfuric acid method (26). Mannan was kept in lyophilized form. Endotoxin-free water was used for yeast culture and mannan extraction, and endotoxin was eliminated from all glassware by dry heat method.

**Scanning Electron Microscopy**

To observe the morphology and microscopic structures of live and HI Candida cells, the samples were prepared for scanning electron microscopic analysis as previously described (27). Briefly, Candida cells were fixed in 2% glutaraldehyde in 0.1 M phosphate buffer at pH 7.2 for 18 h at 4°C. Then, the fixed yeast cells were washed with PBS and deposited on 0.02 μM membrane filter. Post-fixation procedure was performed using 2% osmium tetroxide for 2 h at room temperature. The specimens were dehydrated in a series of ethanol gradients, critical point-dried in CO2 (K850, Quorum Technologies, Laughton, UK), coated with gold (JFC-1200, Jeol, Peabody, MA, USA), and investigated by a scanning electron microscopy (Quanta250, FEI, Hillsboro, OR, USA) with 20,000× and 50,000× magnifications.

**Generation and stimulation of bone marrow-derived dendritic cells (BMDCs)**

Five- to 6-week-old female BALB/c mice were purchased from National Laboratory Animal Center, Mahidol University, and housed at Chulalongkorn University Lab Animal Center. All procedures were approved by the Institutional Animal Use and Care Committee of Chulalongkorn University Lab Animal Center (Protocol number 1573005).

BMDCs were generated as previously described (28). Briefly, bone marrow cells were flushed out from femur and tibia of mice. The cells were seeded in 24-well plates at 1 × 10^6 cells in 1 mL per well, and they were cultured in DC medium containing RPMI 1640 (GIBCO) supplemented with 10% HI fetal bovine serum (GIBCO), 0.2 mM Glutamax (GIBCO), 100 U/mL penicillin and 100 mg/mL streptomycin (HyClone, Logan, UT, USA), 10 ng/mL recombinant murine GM-CSF and 10 ng/mL recombinant murine IL-4 (Peprotech, Rocky Hill, NJ, USA). The cell culture plates were incubated at 37°C with 5% CO2 for 7 days. The media were changed at days 2, 4, and 6.

On day 7, BMDCs were stimulated with HI Candida yeasts or soluble mannan extracted from Candida cell wall for 24-48 h. After stimulation, cultured media were collected for cytokine measurement, and the cells were harvested for investigating the expression of DC maturation markers. Unstimulated BMDCs were used as the negative control, and lipopolysaccharide (LPS)-stimulated BMDCs were used as the positive control.

**Cell viability assay**

To determine cell viability, BMDCs were cultured in 96-well plate at 1 × 10^4 cells/well in 200 μL DC medium. BMDCs were stimulated with HI Candida yeasts or soluble mannan for 48 h. After stimulation, 20 μL of 5 mg/mL MTT 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (Life Technologies/Thermo Fisher Scientific) was added into each well, and the culture plates were incubated in the dark at 37°C with 5% CO2 for 90 min. Dimethyl sulfoxide (DMSO) (Amresco, Solon, OH, USA) was used to dissolve the purple formazan crystals produced by live cells. The optical density (OD) was measured at 570 nm using a microplate reader (Synergy H1, BioTek, Winooski, VT, USA).

**Flow cytometric analysis**

BMDCs were stained with fluorochrome-conjugated anti-mouse CD40, anti-mouse CD80, anti-mouse CD86, anti-mouse I-A/I-E (MHC class II), and anti-mouse CD11c monoclonal antibody (Biolegend, San Diego, CA, USA). The stained cells were assessed using flow cytometry (BD FACSCalibur, BD Biosciences, Franklin Lakes, NJ USA or CytoFlex, Beckman Coulter, Indianapolis, IN, USA) and analyzed by CellQuest Pro software (BD Bioscience) or Kaluza software (Beckman Coulter).

**Cytokine measurements**

The levels of IL-1β, TNF-α, and IL-23 were determined at 24 h after BMDC stimulation, and IFN-γ, IL-12p70, IL-6, IL-23, IL-4, and IL-10 were determined at 48 h after BMDC stimulation. All ELISA was performed according to the manufacturer’s instructions (Biolegend and eBioscience, San Diego, CA, USA).

**Statistical Analysis**

All data obtained in this study were presented as mean ± standard deviation (SD). The statistical differences were determined by one-way ANOVA with post-hoc Tukey HSD test. Significance level was set at P < 0.05.
**Results**

**Effect of Candida yeast cell walls and soluble mannans on DC viability**

To determine the effect of yeast cell walls on DC response without the influence of any secretory molecules from live cells, the yeast cells were inactivated by heat. The morphology and microscopic structures of the yeast cells were examined by a scanning electron microscopy, and it was noted that the heat inactivation method did not disrupt the cell wall integrity (Fig. 1a, b). By using the HI Candida yeasts, the cell wall was expected to be the first component interacting with DCs. To evaluate the cytotoxicity of HI Candida yeasts and soluble mannans in BMDCs, MTT assay was performed. HI C. albicans, C. parapsilosis, and C. dubliniensis significantly reduced DC viability in a DC/yeast ratio dependent manner, and all Candida yeasts showed similar levels of cytotoxicity on BMDCs (Fig. 2).

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**Table 1** Cell viability (mean ± SD%) of BMDCs stimulated with HI Candida yeasts

| Candida species | DC/yeast ratio |
|-----------------|----------------|
|                 | 1:2            | 1:4            | 1:8            |
| C. albicans     | 70.47 ± 6.63*  | 63.67 ± 4.65*  | 55.52 ± 8.23*  |
| C. parapsilosis | 77.76 ± 10.78* | 70.19 ± 3.86*  | 59.12 ± 5.33*  |
| C. dubliniensis | 66.72 ± 3.66*  | 59.25 ± 4.22*  | 56.26 ± 6.21*  |

*P < 0.01 comparison with the negative control (100.00 ± 10.19%).

1*P < 0.05 comparison with ratio of 1:2.

There was no difference among Candida species at the same DC/yeast ratio.

**Table 2** Cell viability (mean ± SD%) of BMDCs stimulated with Candida mannans

| Candida species | Mannan concentration (μg/mL) |
|-----------------|-----------------------------|
|                 | 12.5                        | 25                          | 50                          |
| C. albicans     | 78.16 ± 3.57*               | 75.70 ± 8.60*               | 68.61 ± 10.80*              |
| C. parapsilosis | 76.26 ± 5.12*               | 79.35 ± 3.09*               | 79.83 ± 8.75*               |
| C. dubliniensis | 71.42 ± 7.24*               | 76.83 ± 8.27*               | 73.39 ± 7.87*               |

*P < 0.01 comparison with the negative control (100.00 ± 10.47%).

There was no difference among Candida species at the same mannan concentration.

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**Fig. 1** Morphology and cell wall integrity of live and HI Candida species. Morphology and cell wall integrity of live and HI C. albicans, C. parapsilosis, and C. dubliniensis were observed using a scanning electron microscope with (a) 20,000× and (b) 50,000× magnification. Scale bars as indicated.

**Fig. 2** Cell viability of BMDCs stimulated with HI Candida yeasts and soluble mannans. BMDCs were incubated with HI Candida yeast cells and soluble mannans for 48 h. Then, the cell viability was determined by MTT assay. The various DC/yeast ratios and mannan concentrations were performed as indicated. Unstimulated BMDCs were used as the negative control (-). n = 5; Data represent two independent experiments. Ca, C. albicans; Cp, C. parapsilosis; Cd, C. dubliniensis. The statistical analysis of significant differences is shown in Tables 1 and 2.
Induction of DC activation by Candida yeast cell walls and soluble mannans

To examine the effect of yeast cell wall on DC responses and to avoid undesirable consequences from cell death, the DC/yeast ratio of 1:2 with DC viability above 65% was selected for all subsequent experiments, which was like the effect of mannan at all concentrations (Fig. 2, Tables 1, 2). The DC purity was first confirmed by flow cytometric evaluations of a DC marker, CD11c molecules. The percentage of CD11c+ cells was analyzed by electronic gating on live cells that expressed CD11c (Figs. 3a, 4a). Consistent with the MTT results, the percentage of CD11c+ BMDCs stimulated with HI Candida yeasts and soluble mannans were comparable (Fig. 4a).

DC maturation upon HI Candida yeast and soluble mannan stimulation was observed by an assessment of the expression of activation markers, CD40, CD80, CD86, and MHC class II, on CD11c+ cells (Fig. 3b, 4b-e). BMDCs stimulated with HI C. parapsilosis exhibited a significantly lower level of expression of CD86 and MHC class II, but not CD40 and CD80, when compared with BMDCs stimulated with HI C. albicans and C. dubliniensis. There was no difference in the expression of all activation markers on BMDCs when HI C. albicans was compared with HI C. dubliniensis. On the contrary, when BMDCs were stimulated with soluble mannan at the highest concentration, C. parapsilosis mannan produced the strongest CD40 expression when compared with C. dubliniensis and C. albicans. However, comparable levels of CD80, CD86, and MHC class II expression were observed for C. parapsilosis and C. dubliniensis mannan, and these were notably higher than in BMDCs stimulated with C. albicans mannan. Our findings suggested that the cell wall and soluble mannan of each Candida species differentially induced DC activation. Furthermore, the immunogenicity of the whole yeast cell wall, even with the outermost immobilized mannan layer, was distinct from the soluble mannan.

Functional properties of DCs activated with Candida yeast cell walls and soluble mannans

The proinflammatory cytokines, IL-1β and TNF-α, are primary cytokines detected in oral candidiasis lesion (29-31); therefore, the production of these two cytokines in BMDCs in response to HI Candida and the mannan was compared (Fig. 5). HI C. dubliniensis induced the highest level of IL-1β (Fig. 5a), whereas mannan from all Candida could not promote this cytokine production. In addition, all HI Candida upregulated the expression of TNF-α to comparable levels, but C. parapsilosis mannan induced the highest level of TNF-α (Fig. 5b). Also, at the highest concentration of mannan, BMDCs stimulated with C. albicans mannan significantly produced more TNF-α than those stimulated with C. dubliniensis mannan.

Since the cytokine production from DCs in response to stimuli provided the foundation to direct the type of effector T lymphocytes (32), BMDC secretion of TH cell polarizing cytokines, IFN-γ and IL-12 (Th1), IL-6 and IL-23 (Th17), and IL-4 (Th2) and IL-10 (Treg), when
activated with HI yeast cells or soluble mannans from *C. dubliniensis* induced a weak production of these five cytokines, whereas *C. parapsilosis* mannans induced the highest level of cytokine production (Fig. 4a-d, 4f). Interestingly, neither HI *Candida* yeast cells nor soluble mannans could promote IL-4 production (Fig. 4e). All HI *Candida* yeast cells, but not the soluble mannans, significantly induced the production of IL-10 compared with the negative control. Consistent with the observation of differential induction of DC activation markers, the whole yeast cell wall and soluble mannans differed in the capability for proinflammatory cytokine induction. Altogether, the cell wall and soluble mannans of the NACs triggered a stronger inflammatory response of DC when compared with those of *C. albicans*.

**Discussion**

Oral mucosal DCs play a major role in the induction of immunity and tolerance, and the outcomes of the immune response depend on the functional versatility of these DCs, which in turn depends on the various types of pathogens and stimuli. This study compares the responses of DCs to the cell wall and the soluble mannans of *C. albicans*, *C. parapsilosis*, and *C. dubliniensis*, which are important opportunistic fungi that can cause oral candidiasis. Although mannans are located at the outermost layer of *Candida* cell wall and should be the first component that interacts with host cells, DC responses against...
whole cell wall and soluble mannan are distinct (Figs. 2-4). Candida cell wall comprises mannan, glucan, and chitin, which are located at the outermost, middle, and innermost layer of the cell wall, respectively (13). Thus, the results observed when BMDCs are stimulated with HI yeast cells may be due to the combination of interactions with all components of the cell wall. Furthermore, heat treatment of Candida yeast cells possibly leads to an increased exposure of β-glucan on the cell wall surface and consequently increases the signaling via dectin-1 receptor (17,33).

Our study using the soluble mannan extracted from Candida cell wall provides a more direct evidence for mannan-mediated DC responses. Candida mannan engages with diverse receptors, such as dectin-2, mannose receptor (MR), DC-SIGN, galectin-3, complement receptor 3 (CR3), TLR-2, TLR-4, and TLR-6 (13), depending on its structure (19,21,34). Cell wall mannan of Candida species not only differs in the structure but also proportions of the various structures (22,24,35). These may result in differential DC activation and cytokine production in response to the HI yeasts and the soluble mannan (Figs. 3, 4). Evidence shows that α-mannan of C. albicans binds to dectin-2, whereas β-mannan is recognized by galectin-3 and TLR-2 (13,19,36). It should be noted that C. albicans mannan contains large branches with high content of α-mannan; therefore, the signal transduction via dectin-2 may be dominant (19,35). In contrast, C. dubliniensis contains high level of β-mannan, which possibly engages to galectin-3 (23,37) to activate the TLR-2 signaling pathway. C. parapsilosis mannan contains short branches with low mannosyl content (35,38). It is still unclear which receptor is specific for C. parapsilosis mannan. Nevertheless, a previous study suggests that the recognition of C. parapsilosis and C. albicans cell wall by human peripheral blood mononuclear cells is distinct (38) (Figs. 3, 4).

Studies in a mouse model of oral candidiasis infected with C. albicans shows the induction of IFN-γ, IL-12, IL-6, and IL-23 in oral tissues (39,40). Also, the production of these cytokines from BMDCs stimulated with HI C. albicans (Fig. 4) is seen. Alternatively, while a previous study demonstrated that soluble mannan extracted from C. albicans induced the production of IL-6 and IL-23 (19), the current study finds that C. albicans mannan mainly induced IFN-γ production in a dose-dependent fashion (Fig. 4). These different cytokine induction results may be due to the differences in mannosyl composition of the extracted mannan. While our mannan possibly contains both α- and β-mannosyl linkages, the mannan in the previous study may be composed of only α-mannosyl linkages because yeast cells are cultured in a carbon-limiting, low pH, and low temperature condition where β-mannosyl linkages may not be synthesized (19).

Several reports indicate that β-glucan is the key component provoking protective immunity, and mannan play more of a role in Candida immune evasion (13,41). The observation that C. albicans mannan mediates IFN-γ production may, at least in part, help to explain this.
phenomenon (Fig. 4). Our results suggest that mannan-induced IFN-γ from DCs may skew T cell polarization toward Th1, resulting in the down-modulation of Th17 protective immunity.

To date, there is little evidence on the role of oral immunity on the pathogenesis of *C. parapsilosis* and *C. dubliniensis*. A recent study in a mouse model of *C. parapsilosis* infection demonstrated that the level of proinflammatory cytokines is correlated with disease severity (42). The transcriptome profile in murine macrophage also demonstrates a significant upregulation of proinflammatory cytokine genes, such as TNF-α and IL-1β, in response to *C. parapsilosis* (43). In addition, the same study shows that *C. parapsilosis* promotes high TNF-α production in a human monocyte-derived macrophage model (43). Another recent study using *C. parapsilosis och1* mutant that lacks N-linked mannan in the cell wall implicates *C. parapsilosis* mannan as a virulence factor. Consistently, BMDCs stimulated with soluble mannan of *C. parapsilosis* are highly activated and produce high levels of proinflammatory cytokines but produce a low level of the anti-inflammatory cytokine IL-10 (Figs. 4, 6). In a newborn mouse infection model, the *och1* mutant exhibits less pathogenicity comparing with the wild type *C. parapsilosis* (44). Although the involvement of the high proinflammatory cytokine production induced by *C. parapsilosis* mannan still needs further investigation, the afore-mentioned evidence supports the important role of the cell wall mannan in *C. parapsilosis* immunopathogenicity. Inversely, BMDCs stimulated with *C. dubliniensis* mannan produce only IFN-γ, whereas HI *C. dubliniensis*-stimulated BMDCs produce high levels of both pro- and anti-inflammatory cytokines (Figs. 5, 6). Although the consequence of this result is still unclear, it is possible that HI *C. dubliniensis* induces IL-10, which plays a major role in immune regulation. In addition, *C. dubliniensis* mannan has the lowest potential to induce TNF-α in BMDCs. Altogether, these findings may support previous studies that suggested lower pathogenicity of *C. dubliniensis* (45,46).

Many *Candida* species are dimorphic fungi that can undergo phenotypic switching between the yeast and filamentous forms. Our current work investigated investigates cell wall mannan of *Candida* yeast cells since this form initially colonizes host tissues and the blood stream. Furthermore, *C. parapsilosis* lacks hyphae formation (7); thus, our attention is only focused on the comparison of yeast forms. Nevertheless, it has been demonstrated that cell wall mannan of *Candida* yeast and hyphae differ in composition and structure (47,48), and this difference possibly leads to distinct immune responses against the yeast and hyphae form (29). Thus, further study on DC responses to both yeast and hyphae forms of different *Candida* species are required.

Our results in this study are based on the responses of murine BMDCs, which may be directly applied in understanding mouse models of *Candida* infection. Nevertheless, murine BMDCs may partly differ from human monocyte-derived DCs (MoDCs). Thus, there is a limitation in applying these results to human infection. However, murine BMDCs are widely used as the platform for studying DC immunobiology as well as vaccinology and immunotherapy. In addition, in vitro generated BMDCs are potent APCs that can activate and polarize T cells (28). Moreover, many cells are required for experiments, and this makes the use of human monocytes impossible for this study. Therefore, future work on human DCs is still required to translate the current findings to the human system.

In summary, cell wall mannan may function as a virulence factor promoting the immunopathogenicity and immune evasion of oral *Candida* species through differential activation of DCs. A better understanding of the pathogenesis and induction of protective immunity against various *Candida* species is a necessary foundation for the development of clinical diagnostic tools and therapeutic applications.

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**Conflict of interest**

The authors have no conflict of interest do declare.

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