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Original Article

The critical role of Dectin-1 in host controlling systemic *Candida krusei* infection

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Abstract: There are increasing invasive fungal infections associated with non-albicans, which causes mortal infections in immune deficiency population. *Candida krusei* is a major non-albicans that exhibits intrinsic resistance to fluconazole and makes clinical treatment difficult. Previous studies revealed that C-type lectin receptors (CLRs) Dectin-1 plays critical roles in host defense against *C. albicans* infections. *C. krusei* and *C. albicans* are phylogenetically different although in the same genus. Whether Dectin-1 contributes to host immune response against *C. krusei* infection is still unknown. In the present study, we explored the potential roles of the Dectin-1 in host defense against *C. krusei.* We found that Dectin-1 ligand β-(1,3)-glucan markedly exposed on the cell surface of *C. krusei*, while β-(1,3)-glucan of *C. albicans* is masked. Dectin-1 is required for host myeloid cells recognition, killing of *C. krusei*, and development of subsequent Th1 and Th17 cell-mediated adaptive immune response. Furthermore, Dectin-1-deficient mice (*Dectin-1*$^{-/-}$) are more susceptible to *C. krusei* infection. Together, we confirmed the important roles of Dectin-1 in host defense against *C. krusei* infection, demonstrating a previously unknown mechanism for *C. krusei* infection. Our study, therefore, provides a further understanding of host immune response against *C. krusei*.

Keywords: *Candida krusei*, Dectin-1, immune response, invasive fungal infections

Introduction

Candida albicans is the major pathogen of the *Candida* spp., which causes mucosal and invasive infection [1-3]. However, recent reports suggested that there is an increasing fungal invasive infection caused by non-albicans *Candida* spp [4, 5]. *C. krusei* is one of dominating non-albicans, which displays resistance to fluconazole intrinsically and causes a high mortality rate [6-8]. The morphology and metabolic features of *C. krusei* are different from those of *C. albicans*. *C. krusei* exhibits more hydrophobic than *C. albicans*, rendering it a stronger adhesion to catheters and implants [9, 10]. The key virulence factors are also different between *C. albicans* and *C. krusei*. For example, *C. albicans* can penetrate and invasive host tissue cells by transferring true hyphae from yeast. While *C. krusei* does not form true hyphae as *C. albicans* [10]. Furthermore, although hydrolytic enzymes are considered to be one of major virulence factors in *Candida*, they are not produced by *C. krusei* [10]. These findings suggested that the interaction between *C. krusei* and host immune cells would be probably different from *C. albicans*. Therefore, it would be helpful for clinical treatment to further understand the pathophysiology of *C. krusei* infection.

The imbalance between host defense response and fungal invasion leads to systemic candidiasis development [11]. Pattern recognition receptors (PRRs) including C-type lectin receptors (CLRs), Toll-like receptors (TLRs), dendritic cell-specific intercellular adhesion molecule-3-grabbing non-integrin (DC-SIGN), mannose receptor (MR), complement receptor 3 (CR3), Mincle and Galectin-3 are associated with host recognition of fungal species [12, 13]. Among these PRRs, only mutations in CLR pathway are related to invasive fungal infection in clinical. Previous study has revealed that Dectin-1 mutation (Y238X) patients exhibited a higher

morbidity of mucocutaneous *C. albicans* infections [14].

Previous studies have well characterized host Dectin-1 receptor was involved in the clearance of invasive *C. albicans*, as well as regulation of anti-fungal immune responses [15-17]. Host Dectin-1 senses *C. albicans* invasion by recognizing β-(1,3)-glucan especially [15]. Activation of Dectin-1 signal can initiate a series of anti-fungal responses such as cytokines production, respiratory burst, phagocytosis and the neutrophil extracellular traps formation [18-22]. Dectin-1 signaling is considered to induce Th1 and Th17 responses against *C. albicans* infection. Additionally, Dectin-1 receptors synergizes with TLR2 signaling to defense against of *C. albicans* infection [23-26]. However, the roles of Dectin-1 in host immune response against *C. krusei* remain unclear.

In our present study, we investigated the role of Dectin-1 receptor in host defense against *C. krusei* infection *in vitro* as well as *in vivo*. We found that Dectin-1 is essential for triggering innate immune response and mediating adaptive immune responses mediated by Th cells. We further identified the impact of the Dectin-1 mediated immunity on the pathophysiology of *C. krusei* infection, which provides new insights into host defense against *C. krusei* infection.

Materials and methods

Ethics statement

All animal experiments were performed under the standardized procedures of the "Regulations on the Administration of Laboratory Animals" approved by the State Council of the People's Republic of China. The animal experiment protocol has been verified and approved by the Animal Care and Use Committee of Tongji University (Approval number: TJLAC-016-023).

Mice

Female C57BL/6 mice were purchased from Shanghai Laboatory Animal Center of the Chinese Academy of Sciences. Dr. Gordon D. Brown generously gifted C57BL/6 background Dectin-1-deficient (*Clec7a*$^{-/-}$) mice [27].

Growth conditions of Candida spp

C. albicans SC5314 and *C. krusei* ATCC2159 strains were cultured on sabouraud dextrose agar (SDA) plates and cultured in liquid yeast peptone dextrose (YPD) medium at 30°C for 12-14 h with shaking.

Transmission electron microscopy

After 14 h culture in YPD medium, *C. krusei* ATCC2159 and *C. albicans* SC5314 were harvested and fixed in 5 ml fixative solution at 4°C for 24 h, and then fixed with phosphotungstic acid (1%) and washed with sterile water. With stained by the uranyl acetate, the cells were got through graded alcohol for dehydration and submerged in glycide-ether and propylenoxide. Then a transmission electron microscope (Hitachi H-800) was used to image these thin sections [28].

Fluorescence microscopy

After washed with PBS, *C. albicans* SC5314 and *C. krusei* ATCC2159 yeast cells were blocked with PBS containing with FBS (1%) at 30°C for 2 h. Cy3-labeled secondary antibody was used to culture with the cells which had been 8 h incubated with β-(1,3)-glucan antibody (Biosupplies) at 4°C for β-glucan staining.

Concanavalin A (50 μg/ml, Life Technologies) or Calcofluor white (30 μg/ml, sigma) were used to stain α-mannopyranosyl or chitin for 30 min respectively. After that, the cells were imaged with a laser scanning confocal microscope (TCS SP5; Leica). A flow cytometry (BD FACS Verse) was used to quantify stained cells as described previously [28].

Peritoneal macrophages and neutrophils isolation

We intraperitoneally administrated female mice with 2 mL of thioglycollate (3%, Merck). Neutrophils were harvested 14 h after thioglycollate injection with PBS. After washed with PBS for three times, neutrophils were cultured with RPMI-1640 containing 10% FBS. For macrophages extraction, cells were collected 72 h after thioglycollate injection and cultured with RPMI-1640 containing 10% FBS for 48 h.

Yeast-Macrophage interactions

C. krusei ATCC2159 or *C. albicans* SC5314 yeast cells were subjected to UV-inactivation as described previously [29]. After that, peritoneal macrophages (6×10^6 cells) were chal-

lenged with UV-inactivated *C. krusei* ATCC-2159 in 6-cm plates for the specified time (MOI = 0.1, 1 or 5). For total cell lysate extraction, peritoneal macrophages (3×10^6 cells) were challenged with the UV-inactivated *C. krusei* ATCC2159 in 12-well plates (MOI = 0.1 or 1) for the specified time. For cytokine measurement, peritoneal macrophage (1×10^5 cells) were cultured with UV-inactivated *C. krusei* ATCC2159 or *C. albicans* SC5314 in 48-wells plates for 6 h, following by detecting the cell supernatants.

Western blotting analysis

Lysed peritoneal macrophages were subjected to harvest total lysate and nuclear protein as described previously [30]. After that the protein were subjected to western blotted with relevant antibodies including PCNA, p65, phospho-IκBα, phospho-ErK, JnK, phospho-JnK, p38, phospho-p38, Syk, phospho-Syk, which were purchased from Cell Signaling Technologies.

Cytokine production assay

The concentration of cytokine in cell culture supernatant, peritoneal lavage or murine kidney homogenates were detected by commercial Ready-Set-Go cytokine kits (eBioscience), including IL-17, IFN-γ, IL-6, IL-10, TNF-α, IL-12p40, IL-1β, granulocyte-monocyte colony-stimulating factors (GM-CSF) and chemokine monocyte chemotactic protein-1 (MCP-1).

Neutrophils killing and respiration assay in vitro

Peritoneal neutrophils were harvested and seeded to 48-well plate and challenged with live *C. krusei* ATCC2159 or *C. albicans* SC5314 (MOI = 0.05) as described previously [28]. After 1h incubation at 37°C, the scraped cells were plated on SDA for CFU (Colony-Forming Units) calculation.

To measure the hydrogen peroxide (H_2O_2) production. Neutrophils were challenged with live *C. krusei* ATCC2159 with dihydrorhodamine 123 (10 μM) at 37°C for 1 h. Then the supernatants of cells were subjected to Multiscan Spectrum to measure the dihydrorhodamine 123 conversion for determination H_2O_2 production [30].

Peritoneal C. krusei infection model of mice

Mice were administrated with live *C. krusei* ATCC2159 (1×10^6 cells per mouse) intraperitoneally and sacrificed 4 h post infection. After that, the inflammatory cells were collected and analyzed by flow cytometer (BD FACS Verse™) as described elsewhere [30]. Appropriate antibodies and their parallel isotype controls were used for flow cytometry analysis, including anti-Ly-6C (clone HK1.4, Biolegend), anti-CD11b (clone M1/70, Biolegend), anti-Ly-6G (clone 1A8, Biolegend), Siglec-F (clone E50-2440, BD Pharmingen).

Splenocyte recall response assay

Mice were administered with live *C. krusei* ATCC2159 (5×10^5 cells) cells intravenously. The Mice were sacrificed 7 days post infection, and the splenocytes were harvested and filtered with 200-μm screen. The splenocytes were challenged with *C. krusei* ATCC2159 (MOI = 0.02). Then, the collected splenocytes supernatants were stored at -80°C until cytokines detection.

Murine systemic C. krusei infection model

Mice were administered with live *C. krusei* ATCC2159 (1×10^6 cells) intravenously in 200 μl of 0.9% NaCl. After that, mice were sacrificed 7 days after infection. The weighed livers and kidneys were homogenized with 500 ul of PBS for CFU assay. The homogenized kidneys supernatants were harvested for cytokines detection.

Statistical analysis

We performed more than two biological replicates for all experiments and used two-tailed student's *t*-test with paired analysis to analyze the results of these two groups when appropriate. As for multiple groups analysis, we used one-way ANOVA with Bonferroni post-test analysis. For nonparametrically distributed data, we used either Mann-Whitney or the Kruskal-Wallis test to statistical analysis. Statistical significance was considered when $P < 0.05$.

Results

β-(1,3)-glucan is markedly exposed on C. krusei yeast compared with C. albicans

The cell wall of *Candida* spp. is composed of an outer layer of mannans, β-(1,3)-glucans and

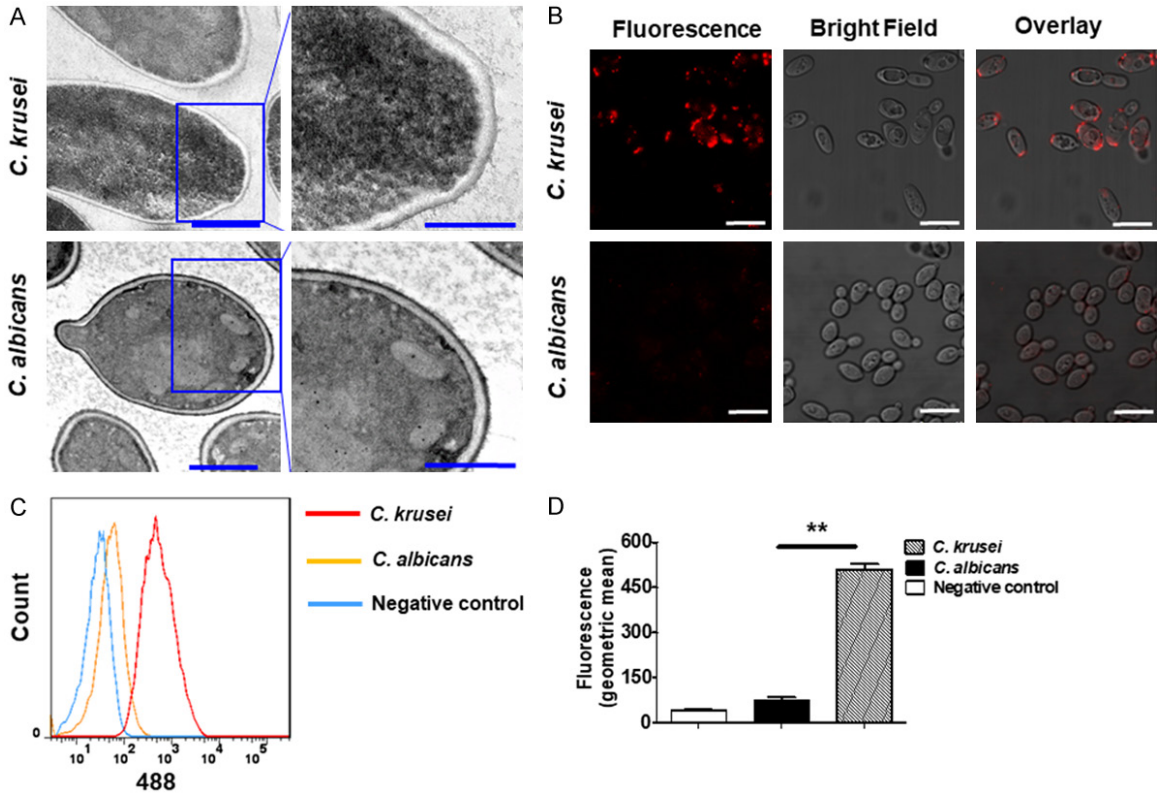

Figure 1. β-(1,3)-glucan is markedly exposed on cell surface of *C. krusei*. A. Representative ultrastructures of *C. krusei* ATCC2159 and *C. albicans* SC5314 cell wall, which were imaged by transmission electron microscopy. B. Representative fluorescence micrographs of *C. krusei* ATCC2159 and *C. albicans* SC5314. The cells were stained with β-glucan antibody and Cy3-labeled secondary antibody for cell surface of β-(1,3)-glucan stained (scale bar = 1 μm). C, D. The cells incubated with β-(1,3)-glucan antibody and subsequently stained with the secondary antibody (Alexa-488-labeled) at 30°C for 1 h. A flow cytometry was used to quantify the fluorescence. Data are representative of 3 independent experiments and shown as means ± SD. **, $P < 0.01$ (one-way ANOVA with Bonferroni's posttest).

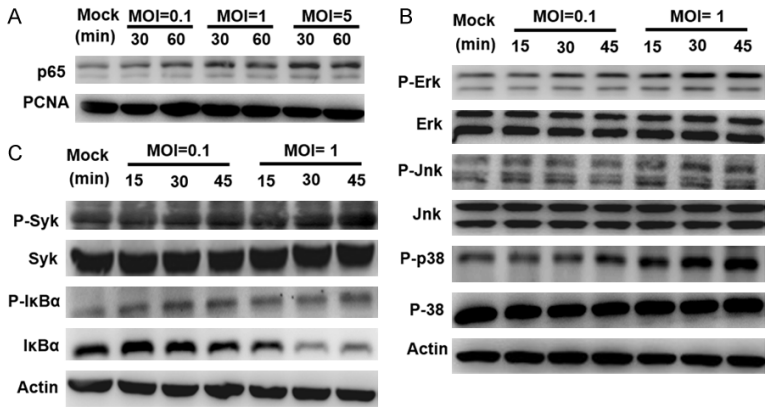

Figure 2. Macrophages displayed NF-κB, MAPK signaling activation upon *C. krusei* challenged. Thioglycolate-elicited peritoneal macrophages were challenged with yeast UV-*C. krusei* ATCC2159 (MOI = 0.1, 1, 5) for the indicated time. The nuclear extracts (A) and cell lysates (B and C) of macrophages were subjected to western blotting analysis with indicated antibodies. Data are representative images of three independent experiments.

chitin, which are the major PAMPs recognized by host PRRs. The differences of cell wall composition will affect the host immune recognition and response to fungal species [12]. As the cell wall structure of *C. krusei* is not well studied as *C. albicans,* we first compared the ultrastructure of *C. krusei* and *C. albicans* cell wall by transmission electron microscopy (TEM) and confocal microscopy. TEM observation revealed that the *C. albicans* β-(1,3)-glucans was surrounded by an external layer of dense mannosylated proteins, while that of *C. krusei* was covered with relatively looser mannosylated proteins (**Figure 1A**). We hypothesized β-(1,3)-glucan may be exposed on the *C. krusei* cell surface. And we subsequently verified our hypothesis with confocal microscopy

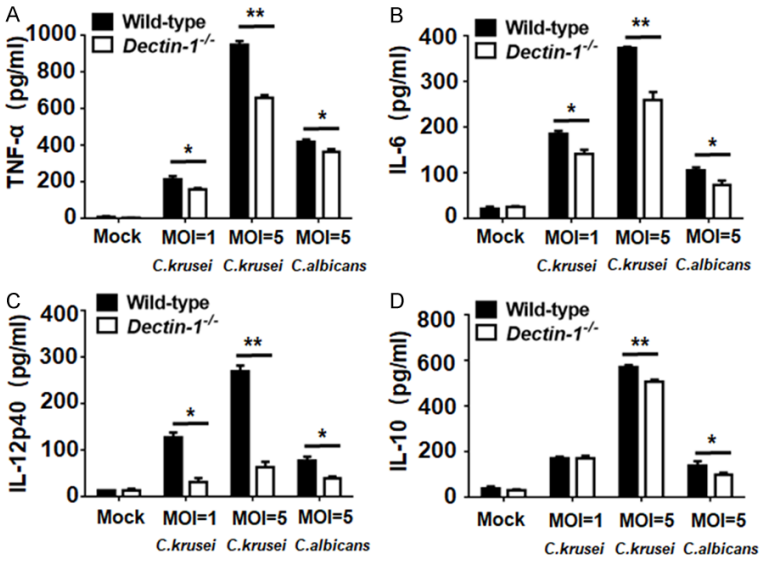

Figure 3. Dectin-1 deficiency affected macrophages recognizing *C. krusei*. ELISA results for the concentration of TNF-α (A), IL-6 (B), IL-12p40 (C) and IL-10 (D) in supernatants of wild-type or *Dectin-1*$^{-/-}$ macrophage stimulated with UV-inactivation *C. krusei* (MOI = 1, 5) and *C. albicans* (MOI = 5) for 6 h. Results are means ± (SD) of representative experiment of three. *, $P < 0.05$, **, $P < 0.01$, ***, $P < 0.001$ (Two-way ANOVA with Bonferroni post-test).

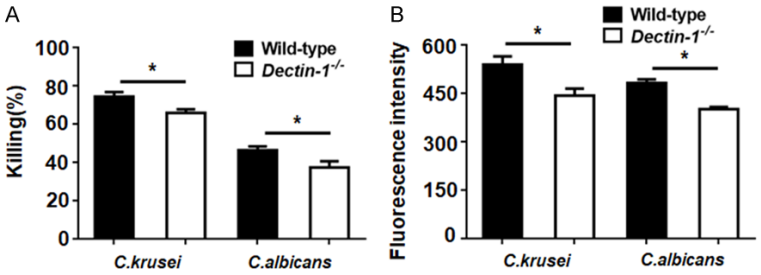

Figure 4. Impaired killing ability of *C. krusei* with respiratory burst of Dectin-1-deficient neutrophils. A. Wild-type, *Dectin-1*$^{-/-}$ neutrophils (6×10^5 cells) were incubated with 1×10^4 unopsonized cells of *C. krusei* or *C. albicans* for 1 h (n = 5). Then the suspension was plated on SDA agar for 48 h to count fungal colonies. B. Peritoneal neutrophils hydrogen peroxide (H_2O_2) production were detected by measuring the conversion of dihydrorhodamine 123 to rhodamine. The fluorescence intensity reflected the amount of dihydrorhodamine 123 translation to rhodamine. Data are representative of 3 independent experiments and shown as means ± SD. *, $P < 0.05$ (Student's t-test).

and flow cytometry observation. And we found that yeast *C. krusei* exhibited markedly β-(1,3)-glucan exposure on the cell surface, while yeast *C. albicans* did not showed significant β-(1,3)-glucan exposure (**Figure 1B-D**). In addition, we found relative lower content of mannan in *C. krusei* than *C. albicans*, while similar content of chitin were detected between *C. krusei* and *C. albicans*. (Figure S1). As it is well accepted that β-(1,3)-glucan is the ligand for Dectin-1 receptor, the exposure of β-(1,3)-glucan on the surface of *C. krusei* indicated that *C. krusei* may be recognized by Dectin-1.

Dectin-1 deficiency affects C. krusei recognition by myeloid cells

Since macrophages are regarded as important detectors of PAMPs [31]. In order to identify the role of Dectin-1 in host against *C. krusei*, we first compared wild-type and Dectin-1-deficient macrophages immune response to *C. krusei* with macrophage-*C. krusei* interaction model. Western blot assay revealed that peritoneal macrophages displayed activation of NF-κB signaling pathway couple with nuclear translocation of p65, Syk phosphorylation, IκBα phosphorylation, accompanied with IκBα degradation (**Figure 2A** and **2C**). Meanwhile, thioglycolate-elicited peritoneal macrophages exhibited MAPK signaling pathway activation with phosphorylation of ErK, p38, and JnK (**Figure 2B**). Additionally, thioglycolate-elicited peritoneal macrophages released inflammatory cytokines, including TNF-α, IL-6, IL-12p40 and IL-10 (**Figure 3**). In comparison, Dectin-1-deficient macrophages showed impaired release of inflammatory cytokines upon both *C. krusei* and *C. albicans* stimulation (**Figure 3**). These results indicated that Dectin-1 plays a nonredundant role for sensing *C. krusei* infection in macrophages.

Since neutrophils are the essential cellular component in inflammatory infiltrates in the tissues to clear *C. albicans* invasion [32], we subsequently challenged Dectin-1-deficient and wild-type neutrophils with *C. krusei* and *C. albicans*. Dectin-1-deficient neutrophils showed attenuated killing ability accompanied by impaired ROS production compared with wild-

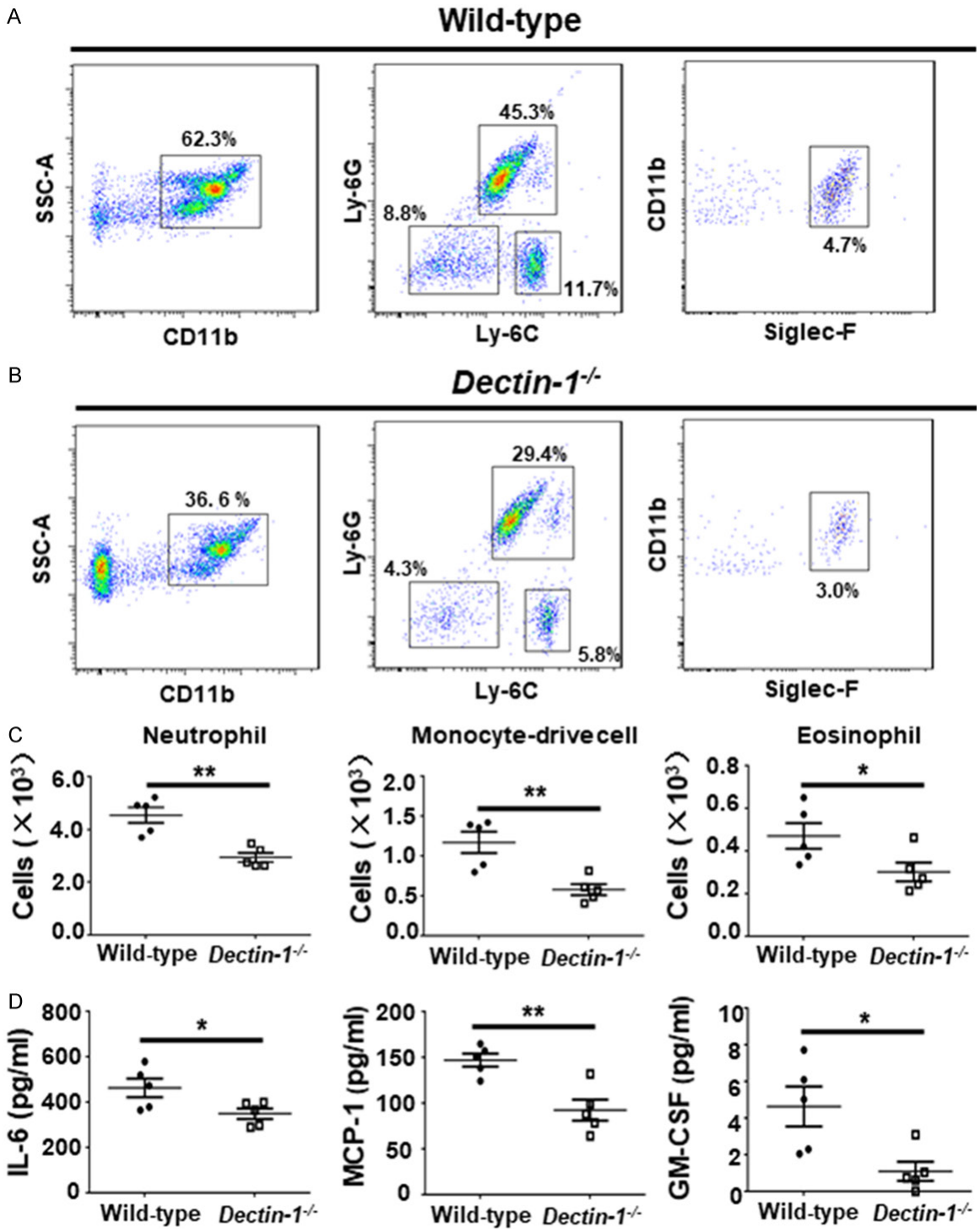

Figure 5. Dectin-1-deficient mice show weaker immune response infected with *C. krusei*. Wild-type and *Dectin-1*$^{-/-}$ mice were administrated intraperitoneally with 5 × 10^5 live *C. krusei* ATCC2195 for 4 h. (A, C) Component analysis of inflammatory cells by flow cytometry including SSC^{high} CD11b$^+$ Ly-6C$^+$ Ly-6G$^+$ neutrophils and SSC^{high} CD11b$^+$ Ly-6$^+$C Ly-6G$^-$ monocyte-derived cells and SSC^{high} CD11b$^+$ Siglect-F$^+$ eosinophils in the peritoneum of the intraperitoneal infection mice(the shown percentages refer to total cells) (n = 5). (B) Scatter plots of myeloid cell subsets in the peritoneum of the indicated mice with intraperitoneal infection (n = 5). (D) ELISAs for lavage fluid cytokines, chemokines and growth factors from the peritoneal cavities. MCP-1, chemokine CCL2; GM-CSF, granulocyte-monocyte colony-stimulating factor (n = 5). Data are representative of 3 independent experiments and shown as means ± SD. * $P < 0.05$; **, $P < 0.01$ (Mann-Whitney non-parametric t-test).

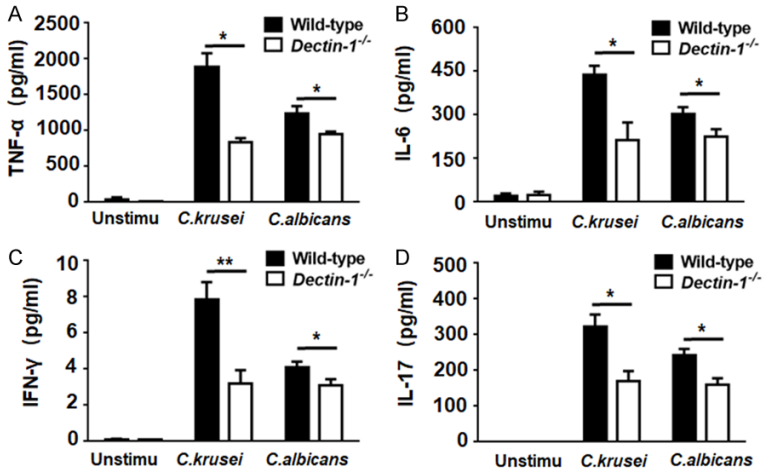

Figure 6. Dectin-1-deficient affected Th cell responses to *C. krusei* infection (splenocytes recall response assay). Wild-type and *Dectin-1*$^{-/-}$ mice were infected with 1 × 10^5 cells of *C. krusei* ATCC2159 or 3 × 10^4 cells of *C. albicans* SC5314 intravenously for 7 days, respectively. And the splenocytes were collected and restimulated with UV- inactivated *C. krusei* for 48 h or 5 days (MOI = 0.02). Accumulation of TNF-α (A), IL-6 (B), IFN-γ (C) for 48 h and IL-17 (D) for 5 days in the supernatants were measured by ELISA (n = 5). Data are representative of three independent experiments and shown as means ± SD. *, $P < 0.05$; **, $P < 0.01$ (Mann-Whitney nonparametric *t*-test).

type neutrophils (**Figure 4**). Therefore, these results suggested that Dectin-1 is necessary for neutrophils recognition and killing of *C. krusei* as well as *C. albicans*.

C. krusei infection in Dectin-1-deficient mice leads to weaker immune response

As our results revealed that Detctin-1-deficient innate immune cells showed impaired activation in myeloid cell-*C. krusei* interaction model, we next used a peritoneal infection model to explore whether the absence of Dectin-1 would affect the host inflammatory responses to *C. krusei*. After 4 h peritoneal infected with *C. krusei*, Dectin-1-deficient mice displayed lower levels of inflammatory cells recruitment including neutrophils (SSC^{high} $CD11b^+$ $Ly\text{-}6C^+$ $Ly\text{-}6G^+$), monocyte-derived cells (SSC^{high} $CD11b^+$ $Ly\text{-}6C^+$ $Ly\text{-}6G^-$), and eosinophils (SSC^{high} $CD11b^+$ $Siglec\text{-}F^+$) compared with wild-type mice (**Figure 5A-C**). At the same time, Dectin-1-deficient mice also exhibited the impaired production of inflammatory cytokine including IL-6, MCP-1 and GM-CSF (**Figure 5D**). Thus, our peritoneal infection model results suggested that the deficiency of Dectin-1 would affect immune response against *C. krusei* development *in vivo*.

Dectin-1-deficient affected Th cells responses to C. krusei infection

After engagement of PRRs on the cell surface, innate immune cells could activate subsequently adaptive immune response by rendering them competent to prime T cells [33]. Since we have found that Dectin-1 participates in the host innate immune response against *C. krusei*, we subsequently explored the role of host Dectin-1 adaptive immunity against *C. krusei*. Wild-type and Dectin-1-deficient mice were intravenously infected with *C. krusei* (1 × 10^5 cells per mouse) or *C. albicans* (3 × 10^4 cells per mouse) and the splenocytes were harvested 7 days post infection and restimulated with UV-inactivated *C. krusei* or *C. albicans* (MOI = 0.01) for 2 or 5 days. Th1 and Th17 responses were measured by detecting the inflammatory cytokine. Our results showed that the splenocytes of Dectin-1-deficient mice produced significantly lower levels of inflammatory cytokine including TNF-α, IL-6, IFN-γ and IL-17 upon *C. krusei* and *C. albicans* challenged. (**Figure 6**), suggesting that Dectin-1 deficient affected Th1 and Th17 responses during *C. krusei* and *C. albicans* infection.

Dectin-1 is required for host sensing C. krusei infection

According to our results that Dectin-1-deficient is required for host immune response to *C. krusei in vitro* and *in vivo*, we subsequently explored whether the Dectin-1-deficiency would affect the susceptibility of *C. krusei* systemic infection. We intravenously infected Dectin-1-deficient and wild-type mice with *C. krusei* ATCC2159 (5 × 10^5 cells per mouse) as a systemic candidiasis model. Our results showed that 7 days post infection, the kidneys and livers of Dectin-1-deficient mice displayed significantly higher fungal burden compared with those in wild-type mice (**Figure 7A** and **7B**). We also found that the cytokines production impaired in the kidney of Dectin-1-deficient mice including

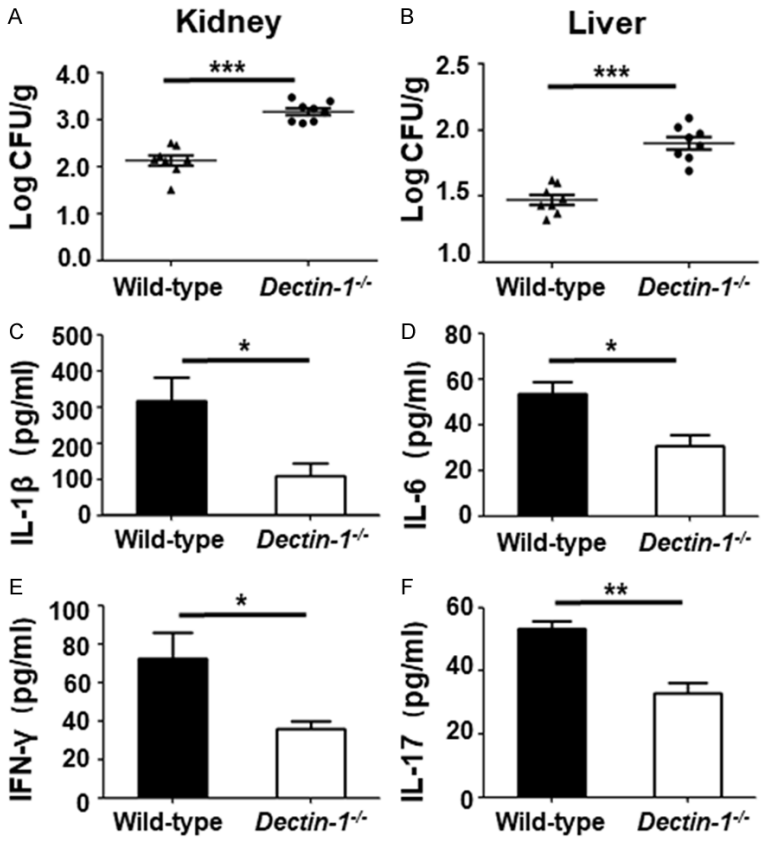

Figure 7. Dectin-1 is required for host sensing *C. krusei* systemic infection. Wild-type mice and Dectin-1$^{-/-}$ mice were administrated intravenously with *C. krusei* ATCC2159 (5×10^5 cells per mouse). (A, B). CFU assay in kidneys and livers of the mice which were infected with *C. krusei* ATCC2159 7 days post infection (n = 8). Data are representative of three independent experiments. Cytokines assay of IL-1β (C), IL-6 (D), INF-γ (E) and IL-17 (F) in kidneys of infected mice (n = 8). Data are representative of three independent experiments and shown as means ± SD. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$ (Mann-Whitney nonparametric *t*-test).

IL-1β, IL-6, IFN-γ and IL-17 compared with wild-type mice (**Figure 7C-F**). Our above results revealed that Dectin-1 plays a nonredundant role for host controlling *C. krusei* systemic infection.

Discussion

Previous studies have demonstrated that recognition of β-(1,3)-glucans by Dectin-1 triggers a series of immune responses and therefore plays an essential role in host defense against *C. albicans* [34, 35]. Unlike *C. albicans, C. krusei* cannot form true hyphal and *C. krusei* displays less virulence fact in terms of lower proteolytic potential than *C. albicans* [9, 10]. Furthermore, the metabolic features of *C. krusei* is also distinguishing with *C. albicans*. Each of these variations would contribute to the diversity of immune response to these Candida species. However, there are few reports on the role of Dectin-1 in the pathophysiology of *C. krusei* infection. In our present study, Dectin-1 was also proven to be a nonredundant receptor defense against *C. krusei*.

The cell wall of fungi varies under the condition of special fungi, morphotype and growth environment, and is the source of PAMPs recognized by immune cells [36]. The main components of *C. albicans* are mannan, β-glucan and chitin [36]. Dectin-1 can recognize β-(1,3)-glucan and initiate a variety of immune response against invasive *C. albicans* [35]. However, *C. albicans* β-(1,3)-glucan of cell wall was masked by cell wall except at the region of budding sites, preventing it recognized by host immune system [37]. Our TEM observation revealed that β-glucan structure of *C. krusei* is surrounded by a dynamic electron-dense outer layer. The outer layer of *C. krusei* is relatively looser than *C. albicans*, and our fluorescence observation found out *C. krusei* yeast cells exhibited a significant β-(1,3)-glucan exposure (**Figure 1A** and **1B**). The exposure of *C. krusei* β-(1,3)-glucan indicated the essential role of Dectin-1 against *C. krusei* infection.

Macrophages are one of the important innate immune cells defensing against invasive fungi [38, 39]. Macrophages can activate several inflammatory pathways including NF-κB and MAPK after interacting with *C. albicans* [40]. In our study, we also found that *C. krusei* can trigger NF-κB and MAPK signaling accompanied by pro-inflammatory cytokines production including IL-6, TNF-α, IL-10, IL-12p40 (**Figures 2** and **3**). TNF-α is an essential cytokine for *C. albicans* immunity by activating neutrophils in the

mouse model as well as involve in controlling intracellular *C. krusei* infection in macrophages [40]. The role of TNF-α has also been confirmed in human, individuals homozygous in TNF-α gene promoter are more resistant to aspergillosis with more TNF-α production [41]. IL-23 (consisting of IL-12p40 and p19) contributes to Th17 differentiation [42]. IL-6 is also associated with the stimulation of Th17 differentiation as well as enhance neutrophils function [43]. Our study showed that Dectin-1-deficient macrophages displayed a significantly lower level of pro-inflammatory cytokines production challenged with both *C. krusei* and *C. albicans*, suggesting an essential role of Dectin-1 for macrophages sensing *C. krusei and C. albicans* infection.

Neutrophils are predominant phagocytes and killers in host clearing *Candida* pathogen, which is the first line of guardians in the innate immune system [32]. After engulfing *C. albicans*, neutrophils can clean them by producing reactive oxygen species (ROS) [32]. Previous report revealed that *C. krusei* display more susceptible upon human neutrophils attacked [10]. We found out Dectin-1-deficient neutrophils displayed a killing and ROS production ability impaired when challenged by *C. krusei* and *C. albicans* (**Figure 4**), suggesting Dectin-1 plays an essential role of host neutrophils killing both *C. albicans* and *C. krusei*. Previous study reported that neutrophils can form neutrophil extracellular traps (NET) to eliminate *C. albicans* [44]. And Dectin-1 deficiency result in impaired NET formation, which may contribute to the impaired killing ability of *C. krusei*. Besides macrophages, monocytes are also vital detectors of PAMPs. They involve in recruiting neutrophils to the fungal infected organs as well as controlling fungal infection [28, 39]. In our peritoneal model, Dectin-1-deficient mice exhibit a less recruitment of neutrophils, monocyte-derived cells and eosinophils, which may result from the lower production of pro-inflammatory cytokines and chemokines including MCP-1, IL-6 and GM-CSF (**Figure 5**). MCP-1 acts vital roles of recruiting monocyte cells, GM-CSF and IL-6 are associated with neutrophils recruitment [45]. These data suggested that the deficiency of Dectin-1 would affect the immune effects against *C. krusei in vivo*.

Immune cells can initiate Th1 and Th17 cells response after engulfing fungal species. Th1 cells are instrumental in the optimal activation of phagocytes at sites of infection and Th17 cells vital in eliminating chronic fungal infection [13]. Previous reports have suggested that Dectin-1 plays a nonredundant role in triggering Th1 and Th17 immune response during *C. albicans* invasion [28]. IFN-γ and Th17 are representative cytokines released by Th1 and Th17 respectively. We also found that the deficient production of IFN-γ and Th17 in Dectin-1-deficient splenocytes restimulated with *C. albicans* and *C. krusei*, suggesting an essential role of Dectin-1 for host Th response against not only *C. albicans* but also *C. krusei* (**Figure 6**).

Our systemic *C. krusei* infection model also revealed impaired production of cytokines including IL-6, IL-1β, IL-17, IFN-γ in Dectin-1-deficient mice (**Figure 7C-F**). IL-17 and IFN-γ can promote neutrophils and macrophages phagocyte and killing of *C. albicans* respectively [43]. IL-1β can enhance Th17 function, which is vital antifungal cytokines. The mutation of IL-1β gene lead to the increased susceptibility to invasive pulmonary aspergillosis infection [43]. At the same time, we found that Dectin-1-deficient mice displayed higher fungal burden in the kidneys and livers compared with wild-type mice. The lower levels of cytokines production of kidney is associated with the higher susceptibility to *C. krusei* systemic infection in Dectin-1-deficient mice (**Figure 7A** and **7B**). These above results suggested Dectin-1 is required for controlling systemic *C. krusei* infection.

In conclusion, our study revealed the roles of Dectin-1 against *C. krusei*. Dectin-1 is required for host myeloid cells recognition and killing *C. krusei in vitro*. Our *vivo* study suggested that Dectin-1 is essential for the innate immune response as well as an adaptive immune response against *C. krusei* infection. Our study revealed an unknown mechanism of Dectin-1 controlling of *C. krusei* infection, thereby provided a further comprehensive of host immunity to *C. krusei*.

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Disclosure of conflict of interest

None.

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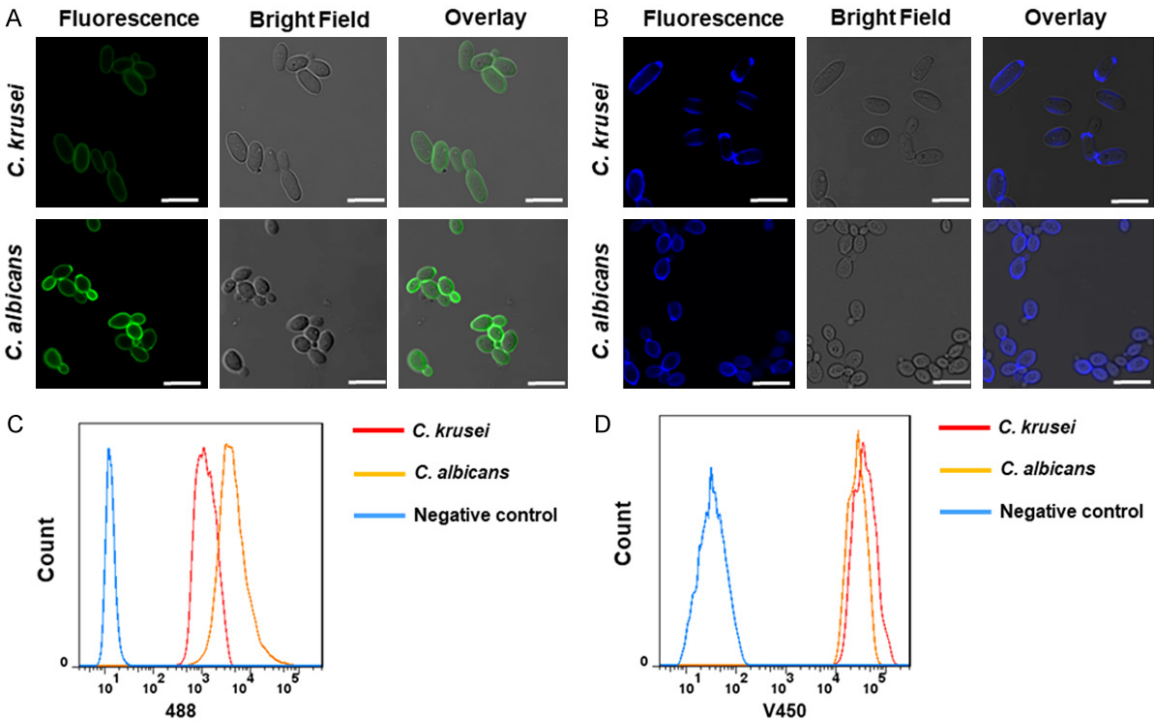

Figure S1. Lower content of mannan in cell wall of *C. krusei* compared to that of *C. albicans*. Representative fluorescence micrographs of cell wall carbohydrate layers from *C. krusei* ATCC2159 and *C. albicans* SC5314, which were stained with ConA-FITC calcofluor white to observe mannan (A), to visualise chitin (B). Scale bar indicated 10 μM. The fluorescence intensity was quantified by flow cytometry [mannan (C), chitin (D)].