Role of Dectin-2 for Host Defense against Systemic Infection with Candida glabrata

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Although Candida glabrata is an important pathogenic Candida species, relatively little is known about its innate immune recognition. Here, we explore the potential role of Dectin-2 for host defense against C. glabrata. Dectin-2-deficient (Dectin-2−/−) mice were found to be more susceptible to C. glabrata infections, showing a defective fungal clearance in kidneys but not in the liver. The increased susceptibility to infection was accompanied by lower production of T helper 1 (Th1) and Th17-derived cytokines by splenocytes of Dectin-2−/− mice, while macrophage-derived cytokines were less affected. These defects were associated with a moderate yet significant decrease in phagocytosis of the fungus by the Dectin-2−/− macrophages and neutrophils. Neutrophils of Dectin-2−/− mice also displayed lower production of reactive oxygen species (ROS) upon challenge with opsonized C. glabrata or C. albicans. This study suggests that Dectin-2 is important in host defense against C. glabrata and provides new insights into the host defense mechanisms against this important fungal pathogen.

The opportunistic fungal pathogen Candida glabrata is the second most frequent cause of candidiasis after Candida albicans, accounting for approximately 15 to 25% of Candida infections (1, 2). Similar to other Candida species, C. glabrata resides as a commensal within the normal microbial flora of humans, but it may also cause serious infections in immunocompromised patients. In the last 3 decades, the number of infections due to non-albicans Candida species has increased significantly (3, 4). The current rise in the incidence of invasive C. glabrata infections is largely the result of the combinational increase in immunosuppressive infections and modern prolonged invasive medical interventions (5). Moreover, C. glabrata is of added concern due to the fact that a fraction of clinical isolates have been proven to be resistant to antifungal agents, such as azoles (6). The mortality rate associated with bloodstream infection with C. glabrata is approximately 50% in cancer patients and is even higher in bone marrow transplant patients (7, 8). However, compared to the literature on C. albicans, few studies have investigated the pathophysiology of C. glabrata infections, despite its high morbidity and mortality burden.

Pattern recognition of fungal components and activation of innate immunity are essential steps in the host defense against Candida species (9). The Candida cell wall is mainly composed of multiple layers of carbohydrates, such as mannann, β-glucan, and chitin, which are collectively recognized by C-type lectins, including macrophage mannose receptor (MR), SIGNR-1, Galectin-3, Mincle, Dectin-1, Dectin-2, and the Toll-like receptors. This recognition leads to activation of the host innate immune system. MR recognizes the N-linked mannans of fungal cell walls, SIGNR-1 recognizes branched α-mannans, and Galectin-3 recognizes β-(1,2)-mannans (9–11). It has recently been reported that Dectin-1 (Clec7a) and Dectin-2 (Clec4n) are specific receptors for β-glucans (12) and C. albicans-derived α-mannans (13), respectively.

Dectin-2 is a C-type lectin receptor primarily expressed by macrophages, dendritic cells and neutrophils, with specificity for structures rich in mannose (14, 15). Dectin-2 uses the FcγRy chain to signal via Syk- and caspase recruitment domain family member 9 (Card9)-dependent pathways (16–18). It represents an important receptor for the generation of the Th17-like adaptive immune response, coordinating the Th1-like responses together with Dectin-1 (17). Human Dectin-2 has a mannose recognition EPN motif (15, 19), strengthening the likelihood that Dectin-2 plays an important role in antifungal immunity. Additionally, Saijo et al. have reported that Dectin-2 is the functional receptor for α-mannans and that Dectin-2−/− mice are more susceptible to C. albicans (13). However, little is known regarding the specific role of Dectin-2 for the host defense against C. glabrata.

In this study, we have explored the contribution of Dectin-2 in the pathophysiology of murine C. glabrata systemic infection. We show that the burden of C. glabrata was significantly increased in kidneys of Dectin-2−/− mice, which was correlated with a decreased phagocytosis and killing of the fungus by the innate immune macrophages (as shown by live-cell video microscopy phagocytosis assays) and neutrophils (as shown by the ex vivo phagocytosis and killing assays). Moreover, the adaptive T helper-derived cytokine responses were also considerably affected in Dectin-2−/− mice. In conclusion, our data demonstrate an important role of Dectin-2 for innate immune responses and its signifi-
cant contribution to the adaptive immune responses against C. glabrata.

MATERIALS AND METHODS

Generation of Dectin-2−/− mice. Dectin-2−/− mice were constructed by Ozgene Pty Ltd., Australia, as described previously (20). Briefly, a targeting vector was designed to introduce a conditional mutation into the mouse Clec4n gene (Ensembl version no. ENSMUSG0000023349; http://www.ensembl.org/Mus_musculus/Gene/Summary?db=core&g =ENSMUSG0000023349;r=6:123229843-123247021). The strategy utilizes mutant loxp sites (lox66 and lox71) to enable an inversion of the flanked sequence in response to the expression of Cre recombinase. The recombination event inactivated the gene by switching the position and direction of the transcriptional termination (STOP) signal with Clec4nt exon 2. The lox66 site was inserted upstream from Clec4nt exon 2, and a neomycin selection cassette (PGK-Neo) was targeted into the intron between exon 2 and exon 3. The selection cassette was flanked with FLP recombination target (FRT) sites to enable removal by FpE-mediated recombination. An inverted STOP element and a FRT site were placed downstream from the selection marker. The 5′ and 3′ homology arms of the vector were approximately 6.1 kb and 5.9 kb, respectively. The targeting vector was linearized with Pmel and electroporated into the C57BL/6 embryonal stem (ES) cell line Bruce4. Neomycin-resistant clones were selected and screened by Southern blotting to identify homologous recombinants. Correctly targeted clones were microinjected into BALB/c blastocysts, which were transferred into pseudopregnant C57Bl6F1 foster females. Chimeric mice were obtained and outcrossed to C57BL/6J females to generate ES cell-derived targeted progeny.

Eight- to 12-week-old female Dectin-2−/− (Clec4n−/−) mice on a C57BL/6J background were obtained from a breeding colony at the Central Animal Laboratory, Radboud University Nijmegen Medical Centre. Age-matched C57BL/6J female mice were obtained from Charles River Wiga (Sulzdld, Germany). All mice weighed between 20 and 25 g. The animals were fed standard laboratory chow (Hope Farms, Woerden, The Netherlands) and water ad libitum. Most of the experiments were repeated at least twice with a minimum of five animals per time point. All experimentation conformed to the terms and conditions of the Ethics Committee on Animal Experiments of Radboud University, Nijmegen. Key experiments, as described in the Methods section, were repeated using Dectin-2−/− mice independently, kindly provided by Y. Iwakura (13). These validation studies were performed at the animal facility of the University of Aberdeen.

Candida strains, culture media, and growth conditions. C. glabrata CBS138 (ATCC 2001), a strain described elsewhere (21), was used in all experiments. C. glabrata was routinely grown and maintained on Sabouraud dextrose plates. For inoculum preparation, a single colony was grown in Sabouraud dextrose broth at 29°C for 24 h, with shaking. Cells were washed twice in sterile phosphate-buffered saline (PBS) and counted using a hemocytometer. Cell density was adjusted with PBS to the desired concentration. C. glabrata was heat killed for 30 min at 95°C. Candida albicans ATCC MYA-3573 (UC 820) was grown overnight to generate yeast cells in Sabouraud dextrose broth at 29°C, with shaking. Cells were harvested by centrifugation, washed twice with PBS, and resuspended in culture medium (RPMI 1640 Dutch modification). C. albicans yeast was heat killed for 30 min at 95°C. To generate hyphae, yeast cells were inoculated and grown overnight at 37°C in culture medium adjusted to pH 6.4 with hydrochloric acid. Hyphae were killed by exposure to 95°C for 30 min and resuspended in culture medium to a hyphal inoculum size that originated from 1 × 10^6 CFU/ml.

C. glabrata infection model and fungal burden. A nonlethal experimental model of disseminated candidiasis was used, in which wild-type and Dectin-2−/− mice were injected intravenously, via the lateral tail vein, with C. glabrata (1 × 10^5 CFU/mouse) in a 100 μl volume of sterile pyrogen-free PBS. Mice were monitored daily. For survival studies, groups of 10 mice were followed-up for a period of 28 days. For immunological and histological studies, subgroups of 5 animals were killed on day 3, 7, or 14 postinfection. Tissues were collected and processed for fungal burden and cytokine analysis. To assess the tissue outgrowth of C. glabrata on these days, the liver and kidneys were removed aseptically, weighed, and homogenized in sterile PBS 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 described elsewhere (22). The CFU were counted after 24 h of incubation at 29°C and expressed as log CFU per gram of tissue.

Histopathology. Kidney samples from infected mice were kept in formalin until processed. Sections were dehydrated with xylene, rehydrated through a graded series of ethanol solutions, and stained with hematoxylin and eosin (H&E) or periodic acid-Schiff (PAS) using conventional staining methods. All individual segments were evaluated for the presence and intensity of inflammation, as well as for the presence of fungi. Tissue sections were analyzed with a VisionTek digital microscope (Sakura), using VisionTek Live software.

In vitro cytokine production. Peritoneal macrophages were isolated from mice by injecting 5 ml of ice-cold sterile PBS (pH 7.4) into the peritoneal cavity. After centrifugation and washing, cells were resuspended in RPMI 1640 culture medium containing 1 mM pyruvate, 2 mM l-glutamine, and 50 mg/liter gentamicin. Cells were counted using a Z1 Coulter particle counter (Beckman Coulter; Woerden, The Netherlands), adjusted to 1 × 10^6 cells/ml, and cultured in 96-well round-bottom microtiter plates (Costar, Corning, The Netherlands) at 1 × 10^6 cells/well in a final volume of 200 μl. After 24 h of incubation with different stimuli at 37°C and 5% CO_2, the plates were centrifuged at 1,400 × g for 8 min, and the supernatants were collected and stored at −80°C until cytokine assays were performed.

Spleen cells were isolated by gently passing spleens through a sterile 200 μm filter chamber. After washing with sterile PBS and centrifugation at 4°C (1,200 rpm for 5 min), cells were resuspended in 2 ml RPMI 1640 in the presence of 20% fetal calf serum and counted, and concentrations were adjusted to 1 × 10^5 cells/ml. Cells were cultured in 24-well plates (Greiner, Alphen a/d Rijn, The Netherlands) at 5 × 10^6 cells/well, and different stimuli were added in a final volume of 1,000 μl. Supernatants were collected at two different time points (depending on the cytokine) as follows. After 48 h of incubation at 37°C and 5% CO_2, 500 μl of supernatant per well was collected and stored at −80°C until cytokine assays were performed; thereafter, the plates were further incubated at 37°C and 5% CO_2 for an additional 3 days. The plates were centrifuged at 1,400 × g for 8 min, and the remaining supernatants were collected and stored at −80°C until cytokine assays were performed. The concentrations of mouse tumor necrosis factor alpha (TNF-α), interleukin-1α (IL-1α), and IL-1β were determined by specific radioimmunoassay (RIA). Mouse IL-6, KC, IL-17, gamma interferon (IFN-γ), IL-22, and IL-10 concentrations were measured by commercial enzyme-linked immunosorbent assay (ELISA) kits (Biosource, Camarillo, CA) according to the instructions of the manufacturer.

Phagocytosis and killing of C. glabrata. Peritoneal macrophages and neutrophils were recruited and phagocytosis and killing assays were performed according to a modification of a method described elsewhere (23). C. glabrata/macrophage or C. glabrata/neutrophil ratios of 10:1 were used in the phagocytosis and killing studies. Peritoneal macrophages or neutrophils from groups of 5 C57BL/6J (control) and 5 Dectin-2−/− mice were elicited by an intraperitoneal (i.p.) injection of heat-killed C. glabrata. Cells were collected in separate sterile tubes either 3 days (macrophages) or 4 h (neutrophils) after C. glabrata infection by washing the peritoneal cavity with 5 ml of ice-cold PBS. Phagocytes were centrifuged (for 10 min at 2,250 × g), counted in a Bürker counting chamber, and resuspended in RPMI 1640 Dutch modification (with 20 μM HEPES and
without L-glutamine; ICN Biomedicals) supplemented with 5% heat-inactivated fetal calf serum, 1% gentamicin, 1% t-glutamine, and 1% pyruvate. The processes of phagocytosis and intracellular killing were studied in an adherent monolayer of phagocytes. To create a monolayer of phagocytes, 5 × 10^5 cells in 100 μl of RPMI 1640 were dispensed into the wells of a 96-well flat-bottom plate (Costar) and incubated at 37°C and 5% CO2. Macrophages were allowed to adhere for up to 2 h, but neutrophils for only 30 min before the monolayers were gently washed with culture medium to remove nonadherent cells. The percentage of adherence was calculated as follows: (1 – [number of nonadherent cells/5 × 10^5] × 100. Subsequently, the cells were incubated with 1 × 10^4 CFU C. glabrata, which were opsonized for 30 min at 24°C in modified Eagle’s medium (MEM; Gibco Life Technologies) that contained 2.5% fresh mouse serum (effector/target ratio, 40:1). After 15 min, supernatants were aspirated, and monolayers were gently washed with MEM to remove noningested C. glabrata. The supernatant and well washings that contained the non-ingested Candida were combined and plated in serial dilutions on Sabouraud agar plates. The percentage of phagocytosed microorganisms was defined as follows: (1 – [number of uningested CFU/CFU at the start of incubation]) × 100.

The killing of C. glabrata by phagocytes was assessed in the same monolayers. After removal of the nonphagocytized Candida, 200 μl of culture medium, consisting of Sabouraud in MEM (50%, vol/vol), was added to the monolayers. After 3 h of incubation at 37°C and 5% CO2, the wells were gently detached with a cell scraper and washed with 200 μl distilled H2O to achieve lysis of phagocytes. This procedure was repeated 3 times, after which the pooled washes were adjusted to a final volume of 1 ml with distilled water. Microscopic examination of the culture plates showed that there was an almost complete removal of phagocytes. To quantify the viable intracellular Candida, 10-fold dilutions of each sample were spread on Sabouraud agar plates and incubated at 37°C for 24 h. The percentage of yeast killed by the phagocytes was determined as follows: (1 – [CFU after incubation/number of phagocytized CFU]) × 100. Phagocyte-free incubations of blastocandidia were included as a control for yeast viability.

Live-cell video microscopy phagocytosis assays. Standard phagocytosis assays were performed as previously described (24). Briefly, 6 × 10^5 live C. glabrata yeasts were added to 2 × 10^5 macrophages in μ-Slide 8-well chambers (ibidi GmbH, Germany) immediately prior to imaging. Video microscopy experiments were performed using an Ultra View VoX live-cell imaging system (PerkinElmer) with the environmental control chamber set at 37°C. Images were captured at 1-min intervals for 3 h using an electron-multiplying charge-coupled device (EMCCD) camera. At least two independent experiments were conducted for each mouse strain, and two movies for each mouse were analyzed ([100] × 100. Phagocyte-free incubations of blastocandidia were included as a control for yeast viability.

RESULTS

Increased susceptibility to C. glabrata systemic infection in Dectin-2−/− mice. To determine the role of Dectin-2 in the host defense against C. glabrata, we first compared the susceptibility to infection of Dectin-2−/− and wild-type control mice. Following infection with C. glabrata, both groups of mice were able to effectively control and eradicate the fungus from the liver within 2 weeks (Fig. 1A). In contrast, while wild-type animals restrained the infection in their kidneys, significantly higher C. glabrata loads were observed in Dectin-2−/− mice (Fig. 1B). Similar increases in the fungal burdens were observed in the kidneys of the Dectin-2−/− mice provided by Y. Iwakura (Fig. 1C). Nonetheless, Dectin-2−/− mice were less susceptible to C. glabrata than has been reported for C. albicans, with no mortality recorded in any of the mouse strains during the 28 days following intravenous infection (data not shown). Compared with the results for noninfected mice (Fig. 2A), histopathological analysis identified zones with lymphocytic infiltration suggestive of chronic inflammation in both wild-type control (Fig. 2B) and Dectin-2−/− (Fig. 2C) mice. Sporadic neutrophil granulocytes could be detected (Fig. 2D and E).

To further characterize the role of Dectin-2 for the recognition of C. glabrata, the ex vivo cytokine profile was assessed in peritoneal macrophages of naïve wild-type and knockout mice.
Interestingly, the innate response levels from uninfected Dectin-2−/− macrophages were slightly higher than the response levels of the macrophages isolated from the wild-type animals (TNF-α, IL-6, IL-1β, and IL-10) (Fig. 3A). Only the IL-6 and KC production levels from Dectin-2−/− macrophages upon stimulation with C. albicans heat-killed hyphae were lower than the levels from wild-type macrophages, although the difference reached statistical significance only for KC (Fig. 3A). Regarding the T cell-derived cytokines (IL-10, IFN-γ, and IL-17) produced by the splenocytes of uninfected mice, no differences could be detected between the two groups of mice (Fig. 3B). The IL-17 production levels in the splenocytes from Dectin-2−/− mice were highly variable.

FIG 1 Greater C. glabrata loads in the kidneys of mice during systemic infection. (A and B) Quantification of Candida colonies from liver (A) and kidneys (B) of wild-type (WT) and Dectin-2−/− mice at days 3, 7, and 14 postinfection is shown. Wild-type and Dectin-2−/− mice received 10⁷ CFU of C. glabrata per mouse intravenously. After 3, 7, and 14 days, organs were collected and serial dilutions were plated on Sabouraud solid medium. Results are means ± SEM (n = 10 to 14 mice per group from 3 independent experiments). Significance was determined with the Mann-Whitney U test. Statistically different groups are indicated as follows: *, P < 0.05; ***, P < 0.001. (C) Fungal burdens in the kidneys of wild-type and Dectin-2−/− mice provided by Y. Iwakura. Wild-type or Dectin-2−/− mice received 10⁷ CFU of C. glabrata per mouse intravenously. After 7 days, kidneys were collected and serial dilutions were plated on Sabouraud agar medium. Results are means ± SEM (n = 12 mice per group from two independent experiments). Significance was determined with the Mann-Whitney U test. **, P < 0.01.

FIG 2 Dectin-2 deficiency affects kidney histopathology during systemic infection with C. glabrata. Histopathological assessment of kidneys in uninfected Dectin-2−/− (A), infected wild-type (B), and infected Dectin-2−/− (C) (hematoxylin-and-eosin [H&E]-stained sections) and infected Dectin-2−/− (D and E) (periodic acid-Schiff [PAS]-stained sections) mice. Representative localized lesions and C. glabrata colonies in the kidneys of Dectin-2−/− mice are shown (C, D, and E). All observations of infected kidneys were performed at day 14 after intravenous injection with C. glabrata.
In a subsequent set of experiments, we studied the ex vivo cytokine responses of peritoneal macrophages and splenocytes isolated from mice infected with *C. glabrata*. No differences were detectable between the two groups of mice in most of the macrophage-derived cytokines assayed (intracellular/extracellular IL-1 and IL-1β, IL-6, IL-10, IFN-γ, and IL-17 accumulation were measured by ELISA. Results are means ± SEM (*n* = 8 mice per group). Significance was determined with the Mann-Whitney U test. Statistically different groups are indicated as follows: *, *P* < 0.05; **, *P* < 0.01.

In a subsequent set of experiments, we studied the ex vivo cytokine responses of peritoneal macrophages and splenocytes isolated from mice infected with *C. glabrata*. No differences were detectable between the two groups of mice in most of the macrophage-derived cytokines assayed (intracellular/extracellular IL-1α and IL-1β and extracellular production of IL-6 and KC; data not shown), with the exception of TNF-α at day 14 postinfection (Fig. 4A). Similar to the results for peritoneal macrophages, ex vivo stimulation of splenocytes from *C. glabrata*-infected mice resulted in significantly lower levels of TNF-α production by Dectin-2/-/- deficient cells upon stimulation with either *C. glabrata* (Fig. 4B) or *C. albicans* (Fig. 4C), although dissimilarities were detectable earlier in the course of the infection. No significant differences could be detected in the amounts of the anti-inflammatory cytokine IL-10 produced by either strain. In addition to the responses of the innate cytokines, Th1 and Th17 responses at 7 days postinfection were also significantly reduced in Dectin-2/-/- splenocytes, as mirrored by the low production of IFN-γ and IL-17 (Fig. 4B). The levels of IL-22 were moderately reduced at day 14 postinfection (Fig. 4B). A similar pattern with more pronounced differences was observed when splenocytes from infected mice were restimulated with *C. albicans* yeast (Fig. 4C). As such, Dectin-2 deficiency results in decreased production of protective T helper-derived cytokines.

**Dectin-2/-/- peritoneal macrophages are less efficient in the uptake and killing of *C. glabrata* ex vivo.** The phagocytic clearance of microorganisms, including yeasts, by professional phagocytes is mediated by receptor activation that drives the engulfment and internalization of the pathogen. We therefore assessed the ability of Dectin-2/-/- peritoneal macrophages to recognize, phagocytose, and kill live opsonized *C. glabrata*. Using a classical ex vivo phagocytosis and killing assay (23), wild-type peritoneal macrophages were found to be slightly more efficient in the phagocytosis and subsequent killing of *C. glabrata* than Dectin-2/-/- macrophages (Fig. 5A). We further investigated the migration of peritoneal macrophages toward *C. glabrata* by live-cell video microscopy using a phagocytosis assay described previously (25). Dynamic analysis of individual macrophages, achieved by tracking cells to determine their directionality, distance, and velocity for the first hour of the assay, demonstrated no major differences in migration dynamics between macrophages harvested from wild-type or Dectin-2/-/- animals (Fig. 5B). However, in the same experiment, a complementary analysis revealed a significant difference between the two types of macrophages in terms of the uptake and phagocytosis of live *C. glabrata*. Following 30 min of coincubation with live *C. glabrata*, 51% of the wild-type macrophages had ingested at least one yeast, whereas Dectin-2/-/- macrophages showed a significantly reduced uptake of 34% (Fig. 5C). This significantly reduced uptake capacity was noticeable early during the phagocytosis assay (30 min and 60 min) but not at a later time point (180 min) (Fig. 5C). Additionally, the total numbers of fungal cells phagocytosed by macrophages from Dectin-2/-/- mice were also significantly lower than the numbers phagocytosed by wild-type macrophages at 30 min and 60 min of coincubation (Fig. 5D). Altogether, these results highlight a partial, yet significant role of Dectin-2 in mediating the uptake and...
engulfment of *C. glabrata* by macrophages. In addition, partial effects on fungal killing may also be envisioned, although more studies should be performed to establish that definitively.

**Dectin-2−/− neutrophils are less efficient in phagocytosing and killing *C. glabrata ex vivo***. We next assessed the recognition and killing of *C. glabrata* by neutrophils, pivotal players in the clearance of invading fungal cells from tissues. Neutrophils were harvested from the peritoneal cavity of both wild-type and Dectin-2−/− mice and further subjected to an *ex vivo* phagocytosis and killing assay (23). The phagocytosis of *C. glabrata* by the Dectin-
2−/− neutrophils was considerably less effective than the phagocytosis of C. glabrata by neutrophils of wild-type mice (Fig. 6A). The killing of C. glabrata by the Dectin-2−/− neutrophils was slightly diminished compared with the killing by control neutrophils (Fig. 6A). Importantly, neutrophils from Dectin-2−/− mice showed a defect in secretion of ROS upon interaction with either live opsonized C. glabrata or C. albicans compared with the ROS secretion of wild-type neutrophils (Fig. 6B). Interestingly, the de-
Dectin-2 Immunity to *Candida glabrata*

**DISCUSSION**

Dectin-2 is one of the main pattern recognition receptors for fungal mannans, with recent studies demonstrating an important role for this receptor in host defense against *C. albicans* (13). In the present study, we show that Dectin-2 is also an important component of the host defense against *C. glabrata*. The increased susceptibility of Dectin-2/−/− mice to a systemic infection with *C. glabrata* is associated with a combination of decreased production of protective T helper-derived cytokines and defective phagocytosis and killing of the yeast by both neutrophils and macrophages.

Little is known of the role of the C-type lectin receptor, Dectin-2. A recent study reported that Dectin-2/−/− mice are highly susceptible to a systemic infection with *C. albicans* (13). Moreover, it has been shown that Dectin-2 plays an important role in the induction of protective Th17 responses (17) and that, together with Dectin-3, it forms a heterodimeric pattern recognition receptor for host defense against *C. albicans* (27).

The intracellular signaling induced by Dectin-2 involves activation of CARD9 (28), phospholipase C-γ (29), and protein kinase C-σ (30). Our study demonstrates that Dectin-2 has a nonredundant role for host defense against *C. glabrata*. This is an important observation not only for our improved understanding of *C. glabrata* infection but also for Dectin-2 biology; while Dectin-2 has been suggested to recognize mainly *C. albicans* hyphae (13), its importance for recognition of a *Candida* species that does not usually develop hyphae is a broadening of the biological importance of Dectin-2. The increased susceptibility to *C. glabrata* was demonstrated in two independently generated groups of Dectin-2/−/− mice, reinforcing this conclusion. However, these mice were less susceptible to *C. glabrata* than to *C. albicans*, as reported earlier (13). The increased susceptibility of Dectin-2/−/− mice to *C. glabrata* was apparent through their incapacity to eliminate the fungus from the kidneys, the target organ of disseminated candidiasis (31). Previously reported studies already showed that systemic candidiasis increased the fungal burden in kidneys of Dectin-1−/− mice compared with the fungal burden in kidneys of the wild-type mice (12). The fact that the animals were able to eradicate *C. glabrata* from the liver, in comparison with the results for the kidneys, confirms recent findings concerning organ-specific *Candida* infection and immune responses mediated primarily by neutrophils and monocytes, as well as by various molecular factors (32–34).

Fungal pathogens have developed a number of strategies to improve their chance of survival in the host environment; e.g., *C. albicans* and *Cryptococcus neoformans* either destroy macrophages or escape via nonlytic exocytosis (35, 36). Interestingly, *C. glabrata* engulfed by macrophages does not undergo a morphological transition but survives and multiplies within mammalian macrophages (37, 38), which are ultimately damaged by ingested *C. glabrata* (38). In our study, we assessed the role of Dectin-2 for the phagocytosis and killing of *C. glabrata* and observed that neutrophils and macrophages lacking this receptor were less capable of ingesting and killing the yeast. Using live-cell video microscopy, we found that significantly fewer Dectin-2−/− macrophages took up *C. glabrata*, in accordance with a lower phagocytic index where phagocytosis had occurred. These defects in the uptake and phagocytosis of *C. glabrata* could not be attributed to a defect in mobility. Finally, signaling through C-type lectin receptors induces cytokines and ROS that both activate the spleen tyrosine kinase (Syk)–CARD9–nuclear factor-κB (NF-κB) pathway (39) and, most likely, induce the intraphagosomal damage of fungal cells. In line with a role of the CARD9 pathway, Drewniak et al. showed that human CARD9 deficiency resulted in a selective defect in the host defense against invasive fungal infection, caused by impaired phagocyte killing in unopsonized samples compared with the phagocyte killing in opsonized samples (40). In the present study, Dectin-2−/− neutrophils produced less ROS than wild-type neutrophils after stimulation of cells with either opsonized *C. glabrata* or *C. albicans*, suggesting a role of Dectin-2 in the killing of *Candida* species despite the presence of opsonins.

The induction of proinflammatory cytokines is an important component of anti-*Candida* host defense. Macrophage-derived proinflammatory cytokines like IL-1, KC, or IL-6 activate the re-
cruitment of phagocytes and the phagocytosis and killing of fungi by phagocytes, and mice deficient in these cytokines have an increased susceptibility to systemic candidiasis (41, 42). No major defects in the production of these cytokines were observed in cells isolated from Candida-challenged Dectin-2−/− mice. In contrast, the secretion of cytokines produced by Th1 and Th17 lymphocytes was significantly decreased in Dectin-2−/− mice. IFN-γ is the main product of Th1 lymphocytes, and it has been proven to have a crucial role in antifungal host defense in mice (43). The defective production of IFN-γ by splenocytes of Dectin-2−/− mice may represent one of the mechanisms determining the increased susceptibility to C. glabrata in these mice. Similarly, the Th1 cytokine, IL-17, represents an important component of antifungal host defense through its activation of neutrophils (44), while patients with defective Th17 responses develop chronic mucocutaneous candidiasis (45). The level of IL-17 produced by Dectin-2−/− splenocytes was significantly decreased at day 7 postinfection, suggesting a role for Dectin-2 in regulating splenocyte responses to fungal infection. The defective Th17 responses in Dectin-2−/− mice could therefore contribute to their susceptibility to C. glabrata. These results underline the conclusions of Saijo et al. (13) concerning Dectin-2-mediated immunity to C. albicans. The IL-17 defect in response to C. glabrata observed in our study was, however, less pronounced than that previously reported for C. albicans (13). Moreover, we should observe that the cytokine differences were most pronounced earlier during infection. At later time points during the infection, the picture starts to be biased by the higher fungal burden in the KO mice, which may counterbalance the defective cytokine production capacity.

The known fungal ligands recognized by Dectin-2 are α-mannans (13). Whereas significant research has been conducted on the structure of the cell wall components of C. albicans cell wall, much less is known regarding the cell wall components of C. glabrata. While recognition of mannan of C. glabrata by Dectin-2 is most likely, future studies should define the precise ligand-receptor interaction profile, especially as C. glabrata is less likely to form hyphae than C. albicans. Moreover, both endogenous (T cell) and exogenous (microbial carbohydrate) ligands for Dectin-2 may be important for the generation and maintenance of tolerance or the recognition of carbohydrate structures. Of particular interest is that previous reports have highlighted the ability of Dectin-2 to specifically signal after recognition of the hyphal form of C. albicans (28). The interaction between C. glabrata and Dectin-2 is different, as C. glabrata does not form traditional compartmentalized hyphae. Still, previous reports have described tubes and pseudohypha formation in several clinical isolates of C. glabrata (46), and these might be recognized by Dectin-2.

In conclusion, we demonstrate that the immune responses triggered by Dectin-2 during infection with C. glabrata might link innate immune recognition, phagocytosis, and killing of the fungus with the adaptive T helper-dependent responses. A combination of these mechanisms controls C. glabrata during systemic infection. Deciphering the precise mechanisms responsible for host defense against C. glabrata represents an important step in understanding the pathophysiology of the disease and for the possibility to design future novel immunotherapeutic strategies.

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