Dectin-1 Is Not Required for Controlling *Candida albicans* Colonization of the Gastrointestinal Tract

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*Candida albicans* is normally found as a commensal microbe, commonly colonizing the gastrointestinal tract in humans. However, this fungus can also cause mucosal and systemic infections once immune function is compromised. Dectin-1 is an innate pattern recognition receptor essential for the control of fungal infections in both mice and humans; however, its role in the control of *C. albicans* colonization of the gastrointestinal tract has not been defined. Here, we demonstrate that in mice dectin-1 is essential for the control of gastrointestinal invasion during systemic infection, with dectin-1 deficiency associating with impaired fungal clearance and dysregulated cytokine production. Surprisingly, however, following oral infection, dectin-1 was not required for the control of mucosal colonization of the gastrointestinal tract, in terms of either fungal burdens or cytokine response. Thus, in mice, dectin-1 is essential for controlling systemic infection with *C. albicans* but appears to be redundant for the control of gastrointestinal colonization.

The innate immune system recognizes *C. albicans* and other pathogens via pattern recognition receptors (PRRs) which interact with ligands termed pathogen-associated molecular patterns (PAMPs). Of the four major families of PRRs, the Toll-like receptor (TLR) family is the best studied, with TLR2 and TLR4, among others, being involved in fungal recognition (1). However, anti-fungal immunity appears to be mediated primarily by members of the C-type lectin receptor (CLR) family, including dectin-1 and dectin-2 (7). The β-glucan receptor dectin-1, in particular, plays a nonredundant role in host defense against a number of medically important fungal species, including *C. albicans*, *Aspergillus fumigatus*, and *Pneumocystis jirovecii* (24, 26, 27). Dectin-1 is expressed primarily on myeloid cells (neutrophils, macrophages, and dendritic cells) and is strongly expressed on immune cells in the lamina propria of the gastrointestinal tract (21). In vitro experiments have shown that dectin-1 mediates a number of cellular functions, including phagocytosis, the respiratory burst, and the production of inflammatory mediators, including cytokines, chemokines, and lipids (6). In vivo studies in mice have demonstrated that dectin-1 is vital for host defense in models of systemic candidiasis (26), although this can be fungal strain dependent (6, 24). In humans, dectin-1 deficiency is associated with a predisposition to mucocutaneous candidiasis, as well as increased gastrointestinal (GI) colonization in immunocompromised individuals, highlighting the importance of this receptor in human mucosal defense (9, 20).

Our previous work suggested that dectin-1 was required to prevent GI tract infection with *C. albicans* during systemic infection in mice (26). In these studies, dectin-1 deficiency was associated with increased fungal burdens and gross morphological changes of the GI tract, including enlargement of the stomach and discoloration of the small intestines (26). Here we further investigated the role of dectin-1 in controlling *C. albicans* GI infections in both systemic and oral infections.

**MATERIALS AND METHODS**

**Mice.** Eight- to 12-week-old female C57BL/6 and dectin-1<sup>−/−</sup> mice (26) backcrossed nine times onto a C57BL/6 background were bred and maintained at the Medical Research Facility, University of Aberdeen. Mice were separately housed in groups in individually ventilated cages, unless otherwise indicated, and provided with food and water *ad libitum*. All experiments were repeated at least two times with five or more mice per time point. All experimentation conformed to the terms and conditions of United Kingdom Home Office licenses for research on animals and the University of Aberdeen ethical review committee.

**C. albicans strains, culture media, and growth conditions.** *C. albicans* strains SC5314 (26) and AM2003-013 and AM2003-016 (19) were routinely grown and maintained on yeast extract-peptone-dextrose (YPD) agar (Sigma-Aldrich). For inoculum preparation, a single colony...
was grown in Sabouraud broth (Oxoid) at 30°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 to the desired inoculum level with PBS, and was confirmed by viable cell counts on agar plates.

**Gastrointestinal model.** The gastrointestinal model was essentially as described previously (15) (see Fig. 4A). To reduce the commensal bacterial and endogenous fungal microbiota, mice were provided with sterile water containing 2 mg/ml streptomycin (Invitrogen), 2,000 U/ml penicillin (Invitrogen), and 0.25 mg/ml fluconazole (Enzo) for 3 days and then switched to water containing streptomycin and penicillin for a further 24 h. Mice were then provided with sterile water containing 1 × 10⁷ CFU/ml C. albicans, 2 mg/ml streptomycin, and 2,000 U/ml penicillin for 5 days. After C. albicans exposure, mice were maintained on sterile water containing 2 mg/ml streptomycin, 2,000 U/ml penicillin, and 0.2 mg/ml gentamicin (Invitrogen). In some experiments, no antibiotics were used at any time point. To monitor colonization, stools were collected from individual mice on day 5 postinfection and every 2 days thereafter. Stools were homogenized in 1 ml PBS and serially diluted, and 25 μl of each dilution was plated on YPD agar containing 0.01 mg/ml vancomycin (Sigma) and 0.1 mg/ml gentamicin. Plates were incubated overnight at 37°C under aerobic conditions and fungal content determined by viable cell count and expressed as CFU per g. Mice were sacrificed at days 7, 14, and 21 after exposure to C. albicans. Kidney, stomach, small intestine, cecum, and large intestine samples were harvested and washed 3 times with 1 ml sterile PBS to remove gut contents. Tissue weights were determined, and samples were transferred into tubes containing 0.5 ml PBS, 0.05% (vol/vol) Triton X-100, and complete mini-EDTA-free protease inhibitor cocktail (as per the manufacturer’s instructions [Roche]). The tissues were then homogenized, serially diluted, and plated on YPD as described above. Cell debris was removed from the remaining tissue homogenates by centrifugation at 13,000 rpm for 15 min at 4°C and stored at −80°C for subsequent cytokine analysis.

**Systemic model.** Mice were inoculated intravenously with 2 × 10⁸ CFU C. albicans SC5314 in 100 μl sterile PBS via the lateral tail vein. Mice were monitored daily and were sacrificed at day 3 postinfection or when judged to be moribund. Tissues were collected and processed for fungal burden and cytokine analysis as described above.

**Cytokine analysis.** Cytokine levels were measured using the Bio-Plex Pro Mouse 23-plex kit (Bio-Rad) and analyzed on the Bio-Plex system using Bio-Plex Manager software as per the manufacturer’s instructions. Stored tissue sample homogenate supernatants were thawed and centrifuged for 15 min at 13,000 rpm at 4°C to remove debris. For each sample, 50 μl of undiluted sample was used, and cytokine concentrations were normalized to sample protein concentrations (bicinchoninic acid [BCA] protein assay kit; Pierce).

**MPO activity.** Myeloperoxidase (MPO) activity in stored tissue supernatants was determined using a myeloperoxidase activity assay kit (Abcam) as per the manufacturer’s instructions.

**Bile acid analysis.** Small intestinal contents were centrifuged at 13,000 rpm. Twenty microliters of the supernatant was serially diluted and analyzed for bile acid content using the Diazyme colorimetric total bile acid assay kit as per the manufacturer’s instructions.

**Histology.** Kidney, stomach, small intestine, cecum, and large intestine samples were removed from uninfected mice and infected mice, snap-frozen in OCT (Sakura), and sectioned. Sections were dehydrated with xylene, rehydrated through a series of different ethanol solutions, and stained with hematoxylin and eosin (H&E) or periodic acid–Schiff (PAS) stain using conventional staining methods. All individual segments of the alimentary tract were evaluated for the presence and intensity of inflammation and for the presence of fungi.

**Statistical analysis.** The two-tailed Student t test was used to compare the two groups of mice. The Mann-Whitney test was used to compare nonnormally distributed data, as determined by the D’Agostino-Pearson omnibus normality test. Survival data were assessed by the log rank test.

All statistical analyses were performed with GraphPad Prism software version 5.04.

**RESULTS**

Dectin-1-deficient mice are more susceptible to systemic C. albicans infection. We had previously observed that dectin-1 deficiency correlated with GI tissue colonization during systemic infection with C. albicans (26). To further explore this phenomenon, we systemically infected C57BL/6 wild-type and dectin-1-deficient mice with 2 × 10⁶ CFU C. albicans SC5314. This dose led to significantly enhanced mortality of the receptor-deficient mice, as we had observed previously in the 129SvEv strain background (26) (Fig. 1A). We examined stool fungal burdens over time (Fig. 1B and C) and assessed tissue fungal burdens at day 3 postinfection (Fig. 1D), a time point chosen as it was prior to the onset of mortality in the dectin-1⁻/⁻ mice. Within 2 days postinfection, we observed significantly higher fungal burdens in the stools of the dectin-1-deficient animals, which further increased by day 3. The identity of the fungi in the stools was confirmed as C. albicans by 18S rDNA sequencing (data not shown). Furthermore, stools plated from uninfected control animals showed no growth of fungal colonies (data not shown). Interestingly, although wild-type mice also had C. albicans present in their stools, these fungal burdens did not change during the course of the infection (Fig. 1C). Similarly, on day 3, infected dectin-1-deficient animals displayed significantly higher fungal burdens in the kidney and GI tissues than wild-type animals, similar to what we had observed previously (26) (Fig. 1D). Thus, dectin-1 deficiency allows enhanced C. albicans infection of the GI tract.
Dectin-1-deficient mice display dysregulated cytokine responses in the GI tract following systemic infection. To investigate the mechanism(s) underlying the increased susceptibility of dectin-1<sup>−/−</sup> mice to systemic GI infection, we measured cytokine levels in gastrointestinal tissues (Fig. 2). In the stomachs of infected dectin-1<sup>−/−</sup> mice we observed significantly increased levels of interleukin-6 (IL-6), granulocyte colony-stimulating factor (G-CSF), gamma interferon (IFN-γ), and MIP-1β (CCL4), and in the large intestine and cecum we observed increased levels of G-CSF and KC (CXCL1). Interestingly, in the latter two tissues we also observed significantly reduced levels of tumor necrosis factor (TNF) in the dectin-1<sup>−/−</sup> mice, and there were significantly reduced IL-1α levels in the large intestine. Thus, these data show that dectin-1 deficiency results in dysfunctional cytokine responses in the GI tissues during systemic infection with C. albicans and that dectin-1-mediated cytokine responses to this pathogen are tissue specific.

Enhanced fungal burdens and dysregulated cytokine responses do not correlate with generalized GI tissue inflammation in dectin-1-deficient mice. The increased fungal burdens and dysregulated cytokine responses in the dectin-1<sup>−/−</sup> mice prompted us to examine the GI tissues for signs of pathology (Fig. 3). Surprisingly, we observed no significant differences in inflammatory cell recruitment or pathology in any of the tissues examined (Fig. 3A). Consistent with these observations, no difference in the levels of myeloperoxidase (MPO) activity, a marker of neutrophil influx, was detected in these tissues (Fig. 3B). PAS staining for fungi, however, did reveal localized invasive C. albicans lesions in dectin-1<sup>−/−</sup> mice, which were not observed in the wild-type animals (Fig. 3C and data not shown).

We had previously observed that C. albicans infection of the GI tract resulted in gross morphological changes, including enlarged stomachs and inflamed intestines (26). While we did observe enlargement of the stomach upon infection, more extensive analysis revealed that this phenotype was not limited to dectin-1<sup>−/−</sup> mice and that it did not occur in all infected animals (data not shown). However, the small intestines of dectin-1<sup>−/−</sup> mice macroscopically appeared to be reproducibly more inflamed than those of the wild-type mice, which did not correlate with our histological analysis described above. On closer examination, the contents of the small intestines in dectin-1<sup>−/−</sup> mice appeared more yellow, producing an inflamed appearance (see Fig. S1 in the supplemental material). As dysregulated production of bile salts, which have a green/yellow appearance, has been observed previously in other models of inflammation (5), we examined the level of bile acids in the contents of the small intestine and observed that these were significantly increased in the dectin-1<sup>−/−</sup> mice (Fig. 3D). Thus, these results indicate that dectin-1 deficiency results in an enhanced, but localized, susceptibility to C. albicans infection in the GI tract. In addition, dectin-1 deficiency has broader effects, including dysregulated production of bile salts.

Dectin-1 deficiency does not alter susceptibility to C. albicans colonization of the GI tract during oral infection. As dectin-1-deficient mice were susceptible to GI tract colonization during systemic infections, we investigated the possibility that this CLR was also involved in controlling colonization of these tissues by C. albicans following oral infection. Mice are not normally colonized by this fungal pathogen, so for these experiments, we made use of an established model of GI colonization (15), whereby mice are treated with antibiotics to displace the endogenous microbiota and then orally infected with C. albicans via the drinking water (Fig. 4A). C. albicans colonization levels in the gastrointestinal tract were subsequently monitored by examining fungal burdens in the stools and in the tissues at specific time points (Fig. 4A). The established level of GI colonization (~10<sup>7</sup> CFU/g of stool) was unaffected by the dose of C. albicans administered in the drinking water (see Fig. S2 in the supplemental material).

Initially we obtained extremely variable results with these experiments, where dectin-1 deficiency had no effect or resulted in either significantly higher or lower GI fungal burdens (data not shown; see Fig. S3 in the supplemental material). Notably, we also
observed variation in results between different cages of the same groups of animals (data not shown). As the microbial composition of the GI tract is transferable between mice and influenced by colonization with \textit{C. albicans} \cite{8, 17}, we performed all subsequent experiments with cohoused wild-type and knockout animals. Under these conditions, we reproducibly found no difference in the colonization levels of the dectin-1−/− mice, compared to wild-type animals, in both stool burdens (Fig. 4B) and GI tissue fungal burdens (Fig. 4C) at any of the time points examined. Interestingly, however, in both wild-type and dectin-1−/− mice, dissemination of \textit{C. albicans} to the kidneys was observed, although this had substantially decreased by the end of the experiment in both groups of animals (Fig. 4C). Importantly, none of the animals succumbed to the infection during these experiments, even with increased infective doses (up to $10^8$ CFU/ml) or after extended time periods (over 60 days). Similar results were also obtained in dectin-1-deficient mice in the 129SvEv background (data not shown).

Strains of \textit{C. albicans} have different propensities in their ability to colonize the mucosae, and SC5314, in particular, is a poor colonizer of these tissues \cite{18}. Therefore, to confirm our observations, we also tested the role of dectin-1 using two additional clinical strains of \textit{C. albicans} (AM2003-013 and AM2003-016), which were originally isolated from infected mucosae \cite{19}. However, as we found with SC5314, dectin-1 deficiency had no effect on GI colonization with either of these clinical strains (see Fig. S4 in the supplemental material).

We have recently shown that dectin-1 deficiency can exacerbate colitis and that this phenotype was dependent on the composition of the microbiome \cite{14}. To exclude the possibility that alterations in the microbiome following antibiotic treatment of mice were masking a potential contribution of dectin-1, we also examined GI colonization in mice which did not receive any antibiotics (see Fig. S5 in the supplemental material). In these experiments, \textit{C. albicans} was rapidly cleared from the GI tract, but, as we had observed in the antibiotic-treated mice, there was no effect of dectin-1 deficiency. Interestingly, while C57BL/6 background
mice rapidly cleared the infection, 129/Sv mice retained a low, but persistent, level of infection throughout the course of the experiment (see Fig. S5 in the supplemental material).

Dectin-1 deficiency does not affect cytokine responses or tissue pathology during *C. albicans* GI colonization following oral infection. Given our findings in the systemic model, we also examined cytokine responses and histopathology in the gastrointestinal tissues from orally infected wild-type and dectin-1−/− mice (Fig. 5). Cytokine responses were examined at day 14 postinfection. Although we observed increased levels of cytokines known to be important in controlling fungal infections, including IL-17 and IL-22 (3), particularly in stomach tissue, there were no significant differences between wild-type and dectin-1-deficient animals (Fig. 5A). The one exception was IL-4, which was slightly, but significantly, higher in the stomachs of dectin-1−/− mice. Histological examination of the GI tract also revealed no difference between the two groups, at either day 7, 14, or 21 postinfection (Fig. 5B; see Fig. S6 in the supplemental material). Localized fungal invasion of the stomach, with resulting hyperplasia of the squamous epithelium, was observed in both groups at days 7, 14, and 21 postinfection (see Fig. S7 in the supplemental material). Thus, these data indicate that dectin-1 is not involved in controlling *C. albicans* colonization of the GI tract following oral infection.

**DISCUSSION**

C-type lectin receptors play a central role in host defense, mediating and directing both innate and adaptive antifungal immunity.
Dectin-1, in particular, has an essential role in the defense against a number of fungal pathogens, including *C. albicans* (26). Our previous work characterizing the role of this receptor had indicated that dectin-1 was required for controlling infection of the gastrointestinal tissues during systemic candidiasis (26). Here we confirmed these findings and demonstrated that loss of dectin-1 led to dysregulated cytokine responses and uncontrolled fungal growth in the GI tract at early time points postinfection. These results are consistent with our previously established role for dectin-1 in mediating protective innate responses to *C. albicans*, including cytokine production and fungal uptake and killing (26). Interestingly, whereas we had previously not observed any differences in cytokine levels in infected kidneys at this early time point (day 3) (26), we could show that dectin-1 was involved in regulating cytokine responses in the GI tract, although this was varied and tissue specific. Surprisingly, the dectin-1-dependent defects were observed only in localized lesions and did not correlate with generalized changes in tissue pathology or inflammation (including neutrophil recruitment). However, we did reproducibly observe gross abnormalities of the small intestines of dectin-1−/− animals, which were not reflected in histopathological changes. Rather, these changes correlated with substantially increased production of bile acids; however, the underlying reasons for this require further exploration.

We also examined the role of dectin-1 in gastrointestinal colonization by *C. albicans*, using an oral model of infection. Surprisingly, despite the essential requirement of this receptor during systemic disease, we observed no differences in GI tract colonization between wild-type and dectin-1-deficient animals. Notably, we achieved reproducible results only when the wild-type and dectin-1-deficient mice were housed together in the same cage. When the animals were caged separately, we obtained variable results; in some experiments dectin-1 deficiency conferred susceptibility, whereas in others it conferred enhanced resistance. In fact, we even observed differences between different cages of the same strain. Although not formally demonstrated here, it is likely that this variation is due to differences in the microbiotas of the mice, which is known to be transferrable between animals and to influence colonization by *C. albicans* (8, 17). The lack of an involvement of dectin-1 in controlling GI colonization under

![Graph and images](http://iai.asm.org/ on April 26, 2021 by guest)
steady-state conditions may reflect the expression of dectin-1 in the lamina propria and not the epithelium (22). However, dectin-1 expression can be induced in epithelial tissues (25), and this receptor may play a role under certain inflammatory conditions, such as we have recently shown during colitis (14).

Our results differ from two recent reports which have implicated dectin-1 in the control of C. albicans infections in the GI tract (2, 10). In the first study, mice with dectin-1-deficient macrophages (and neutrophils) presented with increased fungal burdens in the stomach and cecum following oral infection with C. albicans (10). In the second study, dectin-1 was found to contribute to either resistance or susceptibility to infection, depending on mouse strain background, which was related to the dectin-1 isoform expressed in these animals (2, 11). However, the infection models used were significantly different from our own; in both studies, mice were not pretreated with antibiotics and only a single infective dose was administered (2, 10). However, we also found no role for dectin-1 in mice which had not been treated with antibiotics. The differences in susceptibility observed in these experiments may also reflect the lack of cohabiting between experimental groups and/or the different sources of these animals. Indeed, mice from different locations can differ substantially in their microbiota (4).

While our results suggest that dectin-1 is not involved in controlling the colonization of the GI tract, this receptor is required for controlling C. albicans infection at other mucosal sites. In mouse models, dectin-1 is necessary for controlling infection in both the oral and vaginal mucosa (2, 13). Furthermore, in humans, individuals homozygous for a polymorphism which renders them essentially dectin-1 deficient have enhanced susceptibility to chronic mucocutaneous candidiasis (CMC), which affects the skin, nail beds, and oral and vaginal mucosa (9). Importantly, these patients have defects in Th17 responses, which are critical for the control of mucosal Candida infections (12). Consistent with these observations, we did not observe any dectin-1-dependent effects on the production of IL-17 or IL-22 in the GI tract, and it is likely that other CLR isoforms, such as dectin-2, may play the major role in driving these responses in these tissues (16, 23). However, preliminary analysis suggests that dectin-2 is also not involved in controlling GI colonization following oral infection (see Fig. S8 in the supplemental material).

In conclusion, our data demonstrate that the innate responses triggered by dectin-1 play an essential role in controlling C. albicans infection of the GI tract during systemic infection. In contrast, this receptor appears to have little, if any, role in controlling colonization of the GI tract following oral infection with this organism. Elucidating the mechanisms involved in this process remains a priority if we are to fully understand how our immune system controls and responds to colonization by a pathogen which is a significant cause of human morbidity and mortality.

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