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Sampling of *Candida albicans* and *Candida tropicalis* by Langerin-positive dendritic cells in mouse Peyer's patches

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ABSTRACT

Members of the *Candida* genus, including *C. albicans* and *C. tropicalis* are opportunistic fungal pathogens that are increasingly associated with gastrointestinal infections and inflammatory bowel diseases. In healthy populations, however, *C. albicans* and *C. tropicalis* are considered benign members of the mycobiome, and are presumably kept in check by the mucosal immune system. In this study, we demonstrate in mice that *C. albicans* and *C. tropicalis* are sampled by Peyer's patch (PP) dendritic cells (DCs). Uptake into gut-associated lymphoid tissues occurred rapidly and was at least partly M cell-dependent. *C. albicans* and *C. tropicalis* preferentially localized in (and persisted within) a recently identified sub- population of Peyer's patch DCs distinguished by their expression of the C-type lectin receptor, Langerin. This study is the first to identify a subset of PP DCs capable of sampling *Candida* species.

1. Introduction

Although fungal organisms are a minority in the context of the gastrointestinal microbiome, they are important inhabitants of the normal mammalian flora and have the potential to cause disease in certain clinical populations such as in patients taking broad-spectrum antibacterial drugs and immunosuppressive agents [1]. Fungal members of the genus *Candida*, have the potential to be highly virulent opportunistic organisms. Recently, these were demonstrated to be increasingly associated with gastrointestinal infections and inflammatory bowel diseases. For example, during periods of host immune suppression, inflammation or microbiome dysbiosis, *Candida albicans* is able to breach the epithelial barrier and cause invasive inflammatory disease of the intestinal mucosa [2,3]. *C. albicans* is also recognized as one of the dominant colonizers associated with inflammatory bowel diseases (IBD) like Crohn's and Hirschprung-associated enterocolitis [4–9] . Another constitute of the normal flora, *Candida tropicalis* has also been associated with colonic infections in immune compromised patients [10,11].

It has been reported that *C. albicans* interacts with organized gut-associated lymphoid tissues (GALT) such as Peyer's patches (PPs), which are important inductive sites of mucosal responses to commensal microbes [12–19]. Experimentally, Montagnoli and colleagues reported that intragastric (i.g.) delivery of GFP-labeled *C. albicans* resulted in the accumulation of fungi in murine PPs [20]. The investigators demonstrated by flow cytometry that *C. albicans* accumulated within CD11c$^+$ cells, most likely dendritic cells (DCs). However, it was not determined how *C. albicans* traversed the intestinal epithelium nor did the investigators define the subset(s) of DCs that were responsible for sampling *C. albicans*. Bonifazi et al. later demonstrated that yeast and hyphal forms of *C. albicans* activated PP DCs to elicit distinct T-helper Th/Treg responses *in vivo* [21]. We now know that there are phenotypically distinct subsets of DCs within the follicle associated epithelium (FAE) and sub-epithelial dome region of mouse PPs, including a population that we recently identified as being positive for Langerin (CD207) and responsible for sampling yeast-derived glucan particles (GPs) [22–25]. Langerin is of particular interest in the context of the gut as it binds glycan ligands such as β-glucans, mannose, fucose and *N*-

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acetylglucosamines (GlcNAc) and sulfated glycans that are known to be present on the surface of a wide range of fungal organisms [24,26–28]. In fact, Langerin has been reported to recognize *Candida* species *in vitro* [24,26–28].

In this study, we demonstrate that *C. albicans* and *C. tropicalis* are sampled by PP DCs in mice. We found that uptake into PPs was at least partly M cell-dependent. Within PPs, *C. albicans* and *C. tropicalis* preferentially localized within a sub-population of PP $CD11c^{+}$DCs distinguished by their expression of Langerin. We also found that uptake of *C. albicans* was enhanced by co-administration of the TLR4 ligand, LPS. This study is the first to identify a subset of mucosal DCs capable of sampling *Candida* species.

2. Materials and methods

2.1. Mice

Five-week old Swiss Webster and BALB/c female mice were obtained from Taconic Farms (Hudson, NY). B6.129S2-$Cd207^{tm3\,(DTR/GFP)\,Mal}$/J breeder mice were obtained from the Jackson Laboratory (Bar Harbor, ME). B6.129S2-$Cd207^{tm3(DTR/GFP)\,Mal}$/J offspring were genotyped as previously described to verify the presence of the Langerin-DTR-EFGP knock-in and that they were homozygous [29]. Animals were housed under conventional, specific pathogen-free conditions and were treated in compliance with the Wadsworth Center's Institutional Animal Care and Use Committee (IACUC) guidelines.

2.2. Animal use ethics statement

Experiments described in this study that involve mice were reviewed and approved by the Wadsworth Center's Institutional Animal Care and Use Committee (IACUC). The Wadsworth Center complies with the Public Health Service Policy on Humane Care and Use of Laboratory Animals and was issues assurance number A3183-01. Moreover, the Wadsworth Center is fully accredited by the Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC). Obtaining this voluntary accreditation status reflects that Wadsworth Center's Animal Care and Use Program meets all of the standards required by law, and goes beyond the standards as it strives to achieve excellence in animal care and use.

2.3. Depletion of Langerin DCs

B6.129S2-$Cd207^{tm3(DTR/GFP)\,Mal}$/J mice were given two intraperitoneal injections (i.p.) of diphtheria toxin (DT; 1 μg) from *Corynebacterium diphtheriae* (Sigma, St. Louis, MO) separated by 48 h. To verify that DT treatment resulted in depletion of Langerin DCs from PPs, single cell suspensions of PPs were stained with anti- mouse CD11c-APC (eBioscience, San Diego, CA) and $Langerin^{+}$ DCs were EGFP these cells were then subject to flow cytometry as described in [30]. For experiments that did not require the B6.129S2-$Cd207^{tm3(DTR/GFP)\,Mal}$/J mice, we stained for Langerin DCs using the anti-CD207 Alexa fluor 660-conjugated clone RMUL.2 antibody (eBioscience, San Diego, CA).

2.4. Depletion of M-cells

M-cells were transiently depleted in BALB/c mice by the administration of 250 μg of lK22 -5, a rat anti-mouse RANKL monoclonal antibody, every 2 days by the i.p. route for a total of four doses [31]. Control mice were administered phosphate-buffered saline (PBS) pH 7.4, by i.p. injection.

2.5. Labeling of fungal organisms

C. albicans strains ATCC 18804 and SC5314 and *C. tropicalis* strain ATCC 750 were grown for 48 h at 30 °C at 170 rpm in Sabouraud broth. Prior to staining, fungi were washed twice with sterile PBS, pH 7.4. Strains of *Candida* were labeled at a density of 2×108 cells/ml in 0.5 mg/ml of (FITC) fluorescein (Molecular Probes, Grand Island, NY) in 50 mM carbonate–bicarbonate buffer pH 9.5, for 1 h at room temperature [32]. Alternatively, fungi were stained with 1 mg/ml Concanavalin A conjugated with Alexa Fluor 633 (Molecular Probes) in 0.1 M sodium bicarbonate pH 8.3, for 1 h at room temperature. Strains were washed in PBS pH 7.4, until supernatants were clear. Fluorescently labeled fungi were used in CFU determination experiments; it was previously determine that the fluorescent label did not have a detectable effect on fungal survival and proliferation.

2.6. Labeling of β-glucan particles (GPs)

GPs were labeled as previously described [24]. Briefly, GPs were incubated with either (DTAF) 5-(4,6- dichlorotriazinyl) aminofluorescein or Alexa–Fluor 633 (Molecular Probes) in 0.1 M borate buffer pH 8.5, overnight at room temperature (RT). The labeling reaction was quenched by the addition of 1 M Tris base for 30 min at RT. GPs were washed until excess dye was no longer present and supernatants were clear.

2.7. Oral gavage, Peyer's patch collection and immunostaining

Mice were gavaged with 1×10^{8} fungal organisms or GPs with a 22 G × 1.5-in. blunt-end feeding needle (Popper Scientific, New Hyde Park, NY). PPs were harvested at 4 and 24 h post gavage as described [33]. Briefly, after challenge, mice were sacrificed and PPs were harvested for cryosectioning. PP from the ileum were removed and snap-frozen in liquid nitrogen embedded in Tissue-Tek-Optimal Cutting Temperature (OCT) Compound (Sakura Finetek, Torrance, CA, USA). 20 μm serial cryosections were prepared with a Reichert–Jung 2800e Cryostat (Leica, Wetzlar, Germany). Cyrosections were kept at the room temperature for at least 24 h before staining. PPs were stained as previously described in [24].

2.8. Enumeration of C. albicans and C. tropicalis

Mice were gavaged with 1×10^{8} fungal organisms or GPs as described above. Spleens, mesenteric lymph nodes (MLN) and a total of seven PPs (pooled) per mouse were collected and passed through a 70 μm mesh filter (BD Falcon, San Jose, CA) to generate single cell suspensions, which were then serially diluted and plated in triplicate on Sabouraud dextrose agar plates with antibiotics (40 μg/ml streptomycin; 20 U/ml penicillin; 25 μg/ml chloramphenicol; 40 μg/ml gentamicin). The plates were incubated at 30 °C for 24-48 h and colonies were counted. The number of CFUs per tissue was calculated based on the dilution factor. Enumeration of fungi in MLN and spleen was not possible as there were no CFUs detected. A Bruker Daltonik MALDI-TOF MS Microflex LT Biotyper (Billerica, MA) was used to confirm species identification during fungal enumeration of tissue by CFU.

2.9. Administration of LPS and CT

Lipopolysaccharide (0.3 μg/ml) from *Escherichia coli* O111-B4 (Sigma–Aldrich, St. Louis, MO) was resuspended in PBS pH 7.4, and administered to Swiss Webster mice by oral gavage. Thirty min later the animals were gavaged with FITC-labeled *C. albicans*,