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Short communication

TLR2 modulates gut colonization and dissemination of *Candida albicans* in a murine model

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Abstract

Invasive candidiasis often arises from translocation of endogenous yeasts from the gastrointestinal tract to the bloodstream. Here we describe that both wild type and TLR2−/− mice strains, orally administered with *Candida albicans* yeasts, display similar sustained high level of gut colonization when oral antibacterial treatment is present, while removal of antibiotic treatment causes a progressive clearance of yeasts in control but not in TLR2−/− mice. Fungal invasion of internal organs, following immunosuppression of colonized mice, was increased in TLR2−/− mice. These results point out to a role of TLR2 in gut protection against colonization and endogenous invasion by *C. albicans*.
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Keywords: *Candida albicans*; Gut colonization; TLR2; Immunosuppressed mice

1. Introduction

The gastrointestinal (GI) tract is considered the main source of endogenous yeasts and therefore the origin of invasive candidiasis that arises from the translocation of the microorganism from GI mucosa to the bloodstream when the immune system is depressed. In fact, high fungal GI levels represent an important predisposing factor towards acquired invasive *Candida* infections [1]. *Candida albicans* usually colonizes the GI tract in a harmless form, but it behaves as a pathogen performing translocation into internal organs in the setting of chemotherapy-induced neutropenia and GI mucosal damage, as well as in non-neutropenic, critically ill patients, and there is evidence showing identity between *C. albicans* gut isolates and those causing disseminated infections [2,3]. Therefore, as colonization precedes the invasive infectious process, understanding the pathogen- and host-related factors that interplay leading to GI colonization is essential to unravel the pathogenesis of candidiasis, and factors affecting gut colonization may represent a potential alternative approach in the control of candidiasis [4,5].

Dissemination of *C. albicans* to internal organs and ultimately through the bloodstream to cause invasive candidiasis requires translocation of the yeasts through the GI barrier. In animal models, GI translocation can be promoted through various mechanisms, such as (i) modification of the normal GI microbiome equilibrium that favors GI colonization by the pathogen, (ii) deficiencies in host immune defenses (mainly cellular responses), and (iii) alterations of the intestinal mucosal barrier (mucosal injury) [6,7].

In addition, it is well known that Toll-like receptors (TLRs), notably TLR2, play an essential role in *C. albicans* recognition by the immune system cells and, consequently, in the development of a protective immune response against infection, basically by production of proinflammatory cytokines that are critical for triggering innate immune responses and modulate adaptive immunity [8,9]; TLRs are also critical

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for maintenance and protection of the integrity of the GI mucosa against injury [10,11]. In the present work we have studied the involvement of TLR2 in GI-tract colonization by *C. albicans* and fungal translocation to internal organs in a murine model of long-term high-level sustained colonization using wild type and TLR2−/− mice.

2. Materials and methods

2.1. Mice used

This study was carried out in strict accordance with the recommendations of the "Royal Decree 1201/2005, BOE 252" for the Care and Use of Laboratory Animals, of the "Ministry for the Presidency", Spain. The protocol was approved by the Committee on the Ethics of Animal Experiments of the University of Valencia (Permit Number: A1264596506468). C57BL/6J mice purchased from Harlan Ibérica (Barcelona, Spain) and TLR2 knockout mice (C57BL/6 background) kindly provided by Dr. S. Akira (University of Osaka, Japan) were bred and maintained under specific pathogen-free conditions at the University of Valencia animal facilities. Eight to ten weeks old mice were used in all assays. Assays were performed with 6−10 animals, as indicated in the Results section.

2.2. Yeast strain used and gut colonization assays

The *C. albicans* CAF2 strain was used in this study. The strain was routinely grown at 28 °C in YPD medium (2% glucose, 2% peptone and 1% yeast extract) up to the late exponential growth phase; yeast cells were collected by centrifugation (2000 g, 5 min), suspended in sterile phosphate saline buffer (PBS) at the desired concentration (10^8 yeast cells/mL) and maintained overnight at 4 °C, as described elsewhere [9]. The colonization assay was performed as previously described [4,5]. Briefly, the antibiotic treatment was administered in sterile drinking water *ad libitum*, containing 2 mg/mL streptomycin (Sigma), 1 mg/mL bacitracin (Sigma) and 0.1 mg/mL gentamicin (Sigma). Antibiotic pretreatment started four days (day-4) prior to administration by a single gavage of 10^7 yeast cells in 100 μL PBS (day 0), and maintained during the assays. Fresh stool samples were periodically collected (every 2−4 days) from each mouse and mechanically homogenized in PBS to quantify the fungal burden by determination of colony forming units (CFUs) on Saboureaud-dextrose agar plates added with chloramphenicol and gentamicin (BioMerieux) to inhibit bacterial growth.

2.3. Immunosuppression and fungal translocation assays

C. albicans colonized mice, as above described, were given three oral doses of 100 mg/kg of cyclophosphamide (CPA) in 100 μl PBS on days 6 and 4 and 2 prior to sacrifice by cervical dislocation. Fungal burden in internal organs (liver and kidney) was quantified by colony forming units (CFUs) determination on Saboureaud-dextrose agar plates added with chloramphenicol and gentamicin, following standard procedures previously described [9].

3. Results

3.1. GI colonization by C. albicans is modulated by TLR2

Pretreatment and maintenance of oral administration of antibacterial antibiotics to mice was used to cause a sustained GI-tract colonization by *C. albicans*. As expected, high level and sustained gut colonization by *C. albicans* was observed in antibiotic-treated mice following oral administration of yeasts (Fig. 1). Colonization was detected early (day 1) after *C. albicans* ingestion and maintained stable without additional administration of viable yeast provided that antibacterial antibiotics were ingested with drinking water. Colonization levels were quite similar in both, control C57BL/6 and TLR2−/− mice. Although data suggest that TLR2−/− mice appear to contain an increased fungal burden in feces, differences were not statistically significant. After antibiotic removal, the fungal loads decreased about 10-fold in the TLR2 knockout mice, but they kept stable still at high levels (about $3-4 \times 10^5$ CFU/mL) at least for 18 days; while in control mice *C. albicans* colonization dropped continuously displaying a decrease in fungal burden of about 3000-fold after 18 days. Interestingly, in both mouse types previous *C. albicans* colonization levels were recovered once antibiotic treatment was again applied.

3.2. TLR2 deficiency favors disseminated infection from GI-colonized mice

To favor translocation from GI tract to internal organs, colonized mice were immunosuppressed by administration of CPA. Levels of disseminated infection were measured as fungal burden in liver and kidney. Results showed the presence of viable yeasts in both organs, and significant higher levels of fungal infection in TLR2−/− mice (about 4-fold, compared to C57BL/6 mice, Fig. 2). Both types of mice showed similar levels of viable yeasts in feces prior to determination of fungal burden in organs (log CFU/g: 6,6 ± 0,4 for C57BL/6 mice, and 6,4 ± 0,3 in TLR2−/− mice). Immunosuppression was found to be required to cause disseminated infection, as fungal burden in internal organs was not detectable in CPA-non treated mice, both in wild type and TLR2−/− animals (not shown).

4. Discussion

C. albicans is a common inhabitant of the human GI tract, where it frequently behaves as a harmless commensal, although alterations in the host immune system may lead to a pathogenic behavior of the fungus [12,13]; as most disseminated infections come from an endogenous origin, high fungal gastrointestinal levels are considered a risk factor towards acquired *Candida* infections, a process that involves the

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