Effect of INT1 Gene on Candida albicans Murine Intestinal Colonization

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Background. Increased intestinal colonization with Candida albicans is believed to be a major factor predisposing immunocompromised and postsurgical patients to systemic candidiasis, although the mechanisms facilitating C. albicans colonization remain unclear. Because previous studies have linked the C. albicans INT1 gene to filament formation, epithelial adherence, and mouse virulence, experiments were designed to evaluate the effect of INT1 on intestinal colonization.

Materials and Methods. Mice were orally inoculated with either the parent strain (CAF2, INT1/INT1), an int1 heterozygote (CAG1, INT1/int1), an int1 homozygote (CAG3, int1/int1), or a reintegrant (CAG5, int1/int1 + INT1), and sacrificed 3 and 7 days later for quantitative analysis of cecal C. albicans.

Results. Following oral inoculation with 10^7 C. albicans, only small numbers of each strain were recovered from the cecal flora of normal mice. However, in mice pretreated with oral antibiotics, cecal colonization of each strain was increased (P < 0.01). In addition, cecal colonization was reduced for all int1 mutant strains compared with the parent strain (P < 0.05). By light microscopy, all four C. albicans strains were easily observed in the ileal lumen as both budding yeast and filamentous forms, although only occasional yeast forms appeared adherent to the intestinal epithelium.

Conclusions. C. albicans readily colonized and replicated in the ceca of antibiotic-treated mice. The presence of two functional copies of INT1 appeared to facilitate C. albicans cecal colonization, suggesting that intestinal colonization may be another virulence factor associated with INT1 and that the gene product may be an attractive target to control C. albicans intestinal colonization.

Key Words: Candida albicans; INT1; intestinal colonization; murine.

INTRODUCTION

Of the approximately 100 species of fungi pathogenic for humans, Candida albicans is the most prevalent species in clinical disease [1, 2]. Patients at highest risk for systemic candidiasis include postsurgical patients, immunosuppressed patients, trauma patients, diabetics, premature infants, and patients infected with the human immunodeficiency virus [1–5]. Large proportions of cases occur in intensive care units, and the overall incidence is 10.2% in surgical intensive care units [6]. Risk factors include neutropenia, vascular catheters, broad-spectrum antibiotics, total parenteral nutrition, hemodialysis, oral mucosal colonization, abdominal surgery, prematurity, damage to the gastrointestinal mucosa, burns, and corticosteroids [2, 3, 5]. During 1990–1992, Candida species represented the sixth most common nosocomial pathogen overall and the fourth most common cause of nosocomial bloodstream infections (7% of all bloodstream infections) at hospitals participating in the U.S. National Nosocomial Infection Survey, with C. albicans accounting for the majority of all isolates [2, 7]. Mortality from systemic candidiasis is high, and ranges from 63 to 85% in untreated patients and from 33 to 54% in those who receive appropriate antifungal therapy (reviewed in [8]). Morbidity is also high, and multiple complications...
frequently accompany candidemia, including meningitis, renal insufficiency, renal failure, endophthalmitis, pulmonary abscesses, endocarditis, pericarditis, and osteomyelitis [5]. Unfortunately, clinical diagnosis of candidiasis is often problematic and approximately 50% of patients with systemic infection have negative blood cultures [8]. Thus, diagnosis of systemic candidiasis is often difficult (and therapy may be withheld due to the difficulty in making the diagnosis), and conventional antifungal therapy is often ineffective. Identification of C. albicans virulence factors may help target molecules that can be exploited for novel diagnostic, prophylactic, and therapeutic modalities.

The pathogenesis of C. albicans is postulated to involve adhesion to host epithelial and endothelial cells, as well as morphologic switching from yeast cells (blastoconidia) to filamentous forms, i.e., germ tubes, pseudohyphae, and hyphae [1, 5, 9]. A number of putative C. albicans virulence factors have been analyzed using gene disruption methods such as the "Ura-blast" technique [10–12]. Using this method, disruption of the INT1 gene has been reported to affect epithelial adhesion (adherence to HeLa cells), filamentous growth (using agar media known to facilitate filamentation), and virulence (mortality in intravenously inoculated mice) [13, 14]. This technique facilitates sequential disruption of both copies of a specific gene, resulting in sets of isogenic C. albicans strains that differ from a parent strain at one locus. Using the Ura-blast method, several members of our research group constructed C. albicans strains with various disruptions/reintegrations in the INT1 gene and reported that this single gene contributes to epithelial adhesion, filamentous growth, and virulence [13, 14]. These conclusions were based on a number of factors including the observation that INT1 expression in Saccharomyces cerevisiae triggered filamentous growth in this relatively nonpathogenic yeast that normally exists only as yeast forms [13]. Furthermore, expression of the INT1 protein (Int1p) in normally nonadherent S. cerevisiae facilitated yeast adhesion to cultured human cervical epithelial (HeLa) cells [14]. Results from assays using strains carrying two, one, or no functional copies of INT1 indicated that Int1p augments C. albicans adhesion to HeLa cells, but Int1p did not appear to be the only adhesin involved in this process [14]. Furthermore, adhesion of strains carrying a single copy of INT1 (CAG5) was similar to the total adhesion of the null strain (CAG3) although treatment with antibodies against Int1p reduced the adhesion of CAG5 and did not reduce the adhesion of CAG3 to the HeLa cells. INT1 also contributed to C. albicans filamentous growth on two solid media that normally induce filamentation, namely, milk–Tween agar and Spider medium; however, there was no obvious association between INT1 and C. albicans filamentation using other liquid and solid media that normally induce filamentous growth, supporting the hypothesis that morphologic switching between budding and hyphal forms involves coordinate regulation of many unlinked genes [15, 16]. In addition to modulating adherence and filamentation, INT1 also attenuated virulence, as demonstrated by altered mortality following intravenous inoculation of mice with C. albicans strains containing no, one, or two functional alleles of INT1 [14].

Because it is generally accepted that increased intestinal colonization is a major factor predisposing high-risk patients to systemic candidiasis [3, 5, 9], experiments were designed to study the effect of INT1 on colonization of C. albicans in the ceca of orally inoculated mice. Mechanisms that modulate colonization remain poorly understood. A better understanding of the factors that influence intestinal colonization of C. albicans may be a critical first step in the prevention of systemic disease in high-risk patients, such as postsurgical patients. Experiments presented herein were designed to study the effect of INT1 on colonization of C. albicans in the ceca of orally inoculated mice. The data indicate that INT1 modulates the colonization of C. albicans in the mouse cecum. INT1 may thus play a role in intestinal colonization of C. albicans.

MATERIALS AND METHODS

C. albicans strains and cultivation conditions. C. albicans CAF2 (INT1/INT1 URA3::ura3::imm434) was obtained from W. A. Fonzi, Georgetown University, Washington, DC [11]. Construction of C. albicans CAG1 (INT1/int1::hisG::URA3-hisG ura3::imm434/ura3::imm434), C. albicans CAG3 (int1::hisG/int1::hisG-URA3-hisG ura3::imm434/ura3::imm434), and C. albicans CAG5 (int1::hisG/int1::hisG::INT1-URA3) was described previously [17]. Characteristics of these strains are reviewed in Table 1. All strains have similar growth rates, i.e., generation times, in broth medium [14] and included one functional copy of URA3 because Ura strains have reduced virulence by virtue of their auxotrophy [18–20]. Stock cultures were maintained at ~80°C in Sabouraud's dextrose broth (Difco Laboratories, Detroit, MI) supplemented with 15% glycerol. For inoculation into mice, stock cultures were plated on minimal medium agar [13, 17] supplemented with 2% dextrose, incubated at 30°C for 48 h, then inoculated into minimal medium dextrose broth, incubated at 30°C with shaking for 18 h, washed, and resuspended in sterile saline. Yeast concentration was determined by hemocytometer and verified by quantitative culture on Sabouraud's dextrose agar incubated 48 h at 30°C. All strains grew as blastoconidia under these conditions.

Experimental treatment of mice. Female 18- to 22-g Swiss Webster mice were purchased from Harlan Sprague-Dawley, Indianapolis, Indiana. Each mouse was orally inoculated (feeding needle) with 10⁶ C. albicans CAF2, CAG1, CAG3, or CAG5 suspended in 0.1 ml sterile saline. Control mice received 0.1 ml saline. Mice were sacrificed 3 days later for quantitative analysis of cecal flora (described below). Because antibiotic therapy is a risk factor for intestinal colonization with C. albicans [3, 21, 22], additional experiments were performed in which mice were treated for 3 days with drinking water containing 1 mg/ml bacitracin (Sigma Chemical Co., St. Louis, MO), 2 mg/ml streptomycin sulfate (Sigma), and 0.1 mg/ml gentamicin sulfate (Sigma), then orally inoculated with C. albicans CAF2, CAG1, CAG3, or CAG5. Mice were sacrificed 3 and 7 days later, with
antibiotics continued for the duration of the experiment. To eliminate cross-contamination of C. albicans strains among the various treatment groups, mice were housed in cages with filter tops and were handled by specially trained personnel. The University of Minnesota Institutional Animal Care and Use Committee approved all protocols and Research Animal Resources guidelines were strictly adhered to at all times.

Characterization of mouse cecal flora. Although C. albicans can colonize all portions of the gastrointestinal tract, colonization is typically maximal in the cecum of the adult mouse, and the cecum is most often used to monitor candida colonization in mice [21, 23–25]. Mice were sacrificed by cervical dislocation and ceca were aseptically excised for quantitative analysis of aerobic/facultative cecal flora. Cecal microbes were enumerated as the viable log per gram wet weight of tissue with contents. The lower limit of assay detection was 3.0 log per gram of cecum. For statistical analysis, mice with no detectable cecal microbes were assigned a value of 3.0. There was considerable animal-to-animal variability. Data were not normally distributed and were analyzed by the nonparametric Kruskal–Wallis test with significance set at \( P < 0.05 \), followed by post hoc testing between groups using the Mann–Whitney test. Statistical analysis was performed using StatView 4.5 (Abacus Concepts, Berkeley, CA).

Ileal histology and C. albicans morphology. In an effort to directly observe C. albicans intestinal colonization, ileal sections (1 cm distal to the cecum) from at least eight antibiotic-treated mice per time point of sacrifice (Table 3) were excised, fixed in glutaraldehyde, embedded in JB-4 merthacrylate (Polysciences Inc., Warrington, PA), cut in 2.0-μm cross sections, stained with toluidine blue, and observed by light microscopy. Using this technique, cecal microbes are polymerized in plastic resin, preventing antifungal movement of microbes during sectioning. Sections were observed for specific location and morphology of C. albicans.

<table>
<thead>
<tr>
<th>Strain and genotype</th>
<th>Phenotype on Milk-Tween agar</th>
<th>Adhesion to HeLa cells</th>
<th>Mouse virulence*</th>
</tr>
</thead>
<tbody>
<tr>
<td>CAF2 INT1/INT1</td>
<td>Extensive hyphae</td>
<td>Wrinkled colonies</td>
<td>Maximal</td>
</tr>
<tr>
<td>(parent strain)</td>
<td></td>
<td>with fuzzy edges</td>
<td></td>
</tr>
<tr>
<td>CAG1 INT1/INT1</td>
<td>Moderate hyphae</td>
<td>Wrinkled colonies</td>
<td>Intermediate</td>
</tr>
<tr>
<td>(heterozygous disruption)</td>
<td></td>
<td>with smooth edges</td>
<td></td>
</tr>
<tr>
<td>CAG3 int1/int1</td>
<td>Few filaments</td>
<td>Smooth colonies</td>
<td>Minimal</td>
</tr>
<tr>
<td>(homozygous disruption)</td>
<td></td>
<td>with smooth edges</td>
<td></td>
</tr>
<tr>
<td>CAG5 int1/int1 + INT1</td>
<td>Moderate hyphae</td>
<td>Wrinkled colonies</td>
<td>Intermediate</td>
</tr>
<tr>
<td>(heterozygous reintegrant)</td>
<td></td>
<td>with smooth edges</td>
<td></td>
</tr>
</tbody>
</table>

* Wrinkled appearance indicative of filamentous growth generally extending beyond the periphery of CAF2 colonies (producing fuzzy edges), but confined to upper portions of CAG1 and CAG5 colonies.

** Mortality in intravenously inoculated mice.

RESULTS

Persistence of C. albicans in ceca of normal mice. In an initial experiment, mice were sacrificed 3 days after oral inoculation with saline or with C. albicans CAF2 (INT1/INT1), CAG1 (INT1/int1), CAG3 (int1/int1), or CAG5 (int1/int1 + INT1). At this time, C. albicans was not detected in the cecum of any mouse (Table 2). All mice appeared to have a normal bacterial flora, i.e., relatively large numbers of anaerobic bacteria and smaller numbers of aerobic/facultative gram-negative bacilli consisting primarily of Escherichia coli, Citrobacter sp., Klebsiella pneumoniae, and Proteus sp., and of aerobic/facultative gram-positive bacteria consisting primarily of Lactobacillus sp., Enterococcus sp., and alpha streptococci.

Persistence of C. albicans in ceca of antibiotic-treated mice. Because C. albicans did not readily colonize the normal mouse cecum, and because C. albicans intestinal colonization is often increased in the antibiotic-treated host, additional groups of mice were pretreated for 3 days with oral bacitracin/streptomycin/gentamicin to eliminate competing intestinal bacteria. Mice were then orally inoculated with C. albicans. Prior to antibiotic administration, bacterial culture of fecal pellets indicated that these mice had no detectable cecal microbes extending beyond the periphery of CAF2 colonies (producing fuzzy edges), but confined to upper portions of CAG1 and CAG5 colonies.

During the experiment, mice were sacrificed by cervical dislocation and ceca were aseptically excised for quantitative analysis of aerobic/facultative cecal flora. Cecal microbes were enumerated as the viable log per gram wet weight of tissue with contents. The lower limit of assay detection was 3.0 log per gram of cecum. For statistical analysis, mice with no detectable cecal microbes were assigned a value of 3.0. There was considerable animal-to-animal variability. Data were not normally distributed and were analyzed by the nonparametric Kruskal–Wallis test with significance set at \( P < 0.05 \), followed by post hoc testing between groups using the Mann–Whitney test. Statistical analysis was performed using StatView 4.5 (Abacus Concepts, Berkeley, CA).

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able yeast and no detectable streptomycin-resistant aerobic/facultative gram-negative bacilli. Mice were sacrificed 3 and 7 days later with antibiotics continued for the duration of the experiment. Strictly anaerobic bacteria were never detected in the cecum of any antibiotic-treated mouse, and aerobic/facultative gram-positive bacteria remained in small numbers of \(4.0\ \log/g\) cecum (Table 3). Antibiotic-resistant aerobic/facultative gram-negative bacilli (capable of persistence in the ceca of mice receiving bacitracin, streptomycin, and gentamicin) were recovered in relatively small numbers on Day 3 but increased in numbers by Day 7; on a given day, these numbers were not statistically different from each other. Thus, in general, the four orally inoculated C. albicans stains were exposed to similar levels of competing bacterial flora throughout the duration of this experiment.

Orally inoculated C. albicans persisted and replicated in the intestinal tract of antibiotic-treated mice, with replication inferred by the relatively large numbers of cecal C. albicans compared with the number \(\left(10^3\right)\) in the oral inoculum (Table 3). On Days 3 and 7 after C. albicans inoculation, the parent strain CAF2 (INT1/INT1) was recovered in largest numbers compared with the other three strains, while recovery of the other three strains showed no significant differences among each other. Throughout these experiments, similar numbers of C. albicans were recovered on the several agar media capable of cultivating this organism, including medium lacking uracil, indicating

### TABLE 3

Cecal Colonization of Antibiotic-Treated Mice Orally Inoculated with \(10^3\) Candida albicans CAF2 (INT1/INT1), CAG1 (INT1/int1), CAG3 (int1/int1), or CAG5 (int1/int1 \+ INT1)

<table>
<thead>
<tr>
<th>C. albicans strain(^a)</th>
<th>Oral antibiotic</th>
<th>Day after oral C. albicans</th>
<th>Aerobic/facultative bacteria</th>
<th>Anaerobic bacteria</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>C. albicans(^b)</td>
<td>Gram-negative</td>
</tr>
<tr>
<td>None</td>
<td>No</td>
<td>3</td>
<td>ND(^c)</td>
<td>4.3 ± 0.4</td>
</tr>
<tr>
<td>CAF2</td>
<td>No</td>
<td>3</td>
<td>ND</td>
<td>3.8 ± 0.4</td>
</tr>
<tr>
<td>CAG1</td>
<td>No</td>
<td>3</td>
<td>ND</td>
<td>4.5 ± 0.3</td>
</tr>
<tr>
<td>CAG3</td>
<td>No</td>
<td>3</td>
<td>ND</td>
<td>4.5 ± 0.6</td>
</tr>
<tr>
<td>CAG5</td>
<td>No</td>
<td>3</td>
<td>ND</td>
<td>4.2 ± 0.5</td>
</tr>
</tbody>
</table>

\(^a\) Experiment represents 3 to 4 mice per treatment group in each of 4 experiments, for a total of 12 to 16 mice per group.

\(^b\) Data represent C. albicans recovered on minimal medium agar without uracil.

\(^c\) None detected.

\(^d\) Decreased compared with corresponding group inoculated with CAF2, \(P < 0.05\).

\(^e\) Decreased compared with corresponding group inoculated with CAF2, \(P = 0.07\).
that the URA3 locus remained stable in vivo. In addition, using specific primers for the INT1 locus [17], the polymerase chain reaction was performed on two or three C. albicans colonies isolated from the cecum of each mouse sacrificed at the conclusion of the experiment, for a total of 118 colonies tested; results were uniformly consistent with the genotype of the inoculated strain, indicating that the INT1 locus remained stable and that no cross-contamination occurred between the groups of mice. Thus, cecal colonization was consistently increased in mice colonized with the parent strain CAF2 containing two functional copies of INT1, while colonization was significantly lower in mice colonized with C. albicans lacking one or both copies of INT1.

It should be noted that the size of the inoculum did not appear to affect the climax cecal population levels of C. albicans. In a preliminary experiment, antibiotic-treated mice (seven per group) were orally inoculated with a relatively large number (10^7) of C. albicans and attained cecal population levels after 48 h (average ± SE log/g) of 5.4 ± 0.3 and 4.2 ± 0.3 for CAF2 (parent strain) and CAG3 (null mutant), respectively. These population levels were not significantly different from those obtained following oral inoculation of 99.99% fewer (i.e., 10^3) C. albicans (Table 2). The lower inoculum was used for the experiments reported in Table 2 to clearly distinguish between colonization of C. albicans (defined as persistence accompanied by in vivo replication) and simple persistence of the inoculated strain.

Ileal histopathology and morphology of C. albicans CAF2, CAG1, CAG3, and CAG5. Nearly all C. albicans CAF2, CAG1, CAG3, and CAG5 were observed in the ileal lumen as budding yeast and filamentous forms (Fig. 1A); only occasional yeast forms appeared adherent to ileal epithelium (Fig. 1B). Despite extensive searching, C. albicans CAG3 (int1/int1) was observed only in the yeast form and filaments were not found; however, due to the relatively low level of CAG3 colonization, it is premature to conclude with certainty that CAG3 did not form filaments in the ileal lumen.

**DISCUSSION**

Factors that influence intestinal colonization of C. albicans remain obscure. Results of experiments presented herein indicated that INT1 facilitated persistence of C. albicans in the ceca of orally inoculated
mice. This effect was evident only in mice with antibiotic-induced alterations in cecal bacteria and not in mice with a normal cecal flora. Without antibiotic treatment, C. albicans was rapidly eliminated from the intestinal tract of mice with a normal cecal flora.

Antibacterial therapy has been repeatedly associated with increased candida colonization in high-risk patients [2, 3, 29] and in experimental animals [22-25]. As expected, C. albicans persistence in the mouse cecum was facilitated by antibacterial treatment. Following oral inoculation with C. albicans CAF2 (parent strain, INT1/INT1), mice treated with bacitracin/streptomycin/gentamicin had an average of $10^{3.4}$/g cecal C. albicans. This concentration was similar to that recovered from the ceca of mice treated with other broad-spectrum antibiotics (clindamycin and gentamicin) and orally inoculated with a wild-type C. albicans strain [21]. Thus, the concentrations of cecal CAF2 observed in this study were similar to what might be expected using wild-type C. albicans.

As expected, similar C. albicans cecal population levels were recovered from antibiotic-treated mice orally inoculated with either $10^7$ or $10^8$ C. albicans. In general, the number of microbes in an oral inoculum does not affect the climax (maximal) intestinal population level of a microbe; rather, the most important factor controlling intestinal colonization seems to be the level of microbial competition. Indirect evidence for this statement is provided by the inability of orally inoculated C. albicans to colonize the ceca of normal mice, yet the same inoculum resulted in substantial cecal colonization of antibiotic-treated mice.

Surprisingly, while cecal colonization was reduced by the loss of one or both copies of INT1, there was no significant difference between strains carrying one copy of INT1 (either as a heterozygote [CAG1] or as a reintegrant [CAG5]) and strains lacking both copies of INT1 (CAG3). This is different from what we observed in intravenously inoculated mice, where CAG3 caused less mortality than CAG1 and CAG5 [17]. We do not know why cecal colonization appears more sensitive to the loss of even one copy of INT1, but we have previously noted a similar effect of loss of one or both copies of INT1 on the ability of C. albicans INT1 mutant strains to adhere to HeLa cell monolayers [17]. Others [30] have also observed a similar gene dosage effect in C. albicans. Apparently, the ability of C. albicans to colonize the mouse cecum is sensitive to the dosage of INT1, and perhaps to the dosage of other genes encoding surface proteins as well.

We are aware of only two other C. albicans genes, namely, FAS2 and MNT1, that have been studied for the ability to modulate colonization in vivo. Zhao et al. [31, 32] noted that disruption of C. albicans FAS2 was associated with decreased oropharyngeal colonization of weanling rats, as well as decreased virulence in intravenously inoculated mice. And, Buurman et al. [30] reported that disruption of either one or both copies of C. albicans MNT1 was associated with decreased vaginal colonization in rats, as well as decreased virulence in intravenously inoculated mice and guinea pigs. Thus, disruptions in INT1, FAS2, and MNT1 have all been associated with decreased colonization as well as attenuated systemic virulence, suggesting that genes associated with colonization may be virulence factors.

Although it has long been speculated that morphogenesis from blastoconidial to hyphal forms might be a virulence mechanism for candida [9, 27], additional studies are needed before concluding that morphologic switching, such as that associated with INT1, plays a role in regulating intestinal colonization of C. albicans. Our light microscopic examinations of ileal sections are inconclusive. Filamentous forms of C. albicans CAF2, CAG1, and CAG5 were all observed in the ileal lumen despite significant differences in cecal colonization. However, assuming morphologic switching does facilitate intestinal colonization, compounds that interfere with proteins expressed during hyphal development might be attractive prophylactic and therapeutic agents to decrease the costly morbidity and mortality associated with systemic candidiasis. This topic is worthy of further study due to the prevalence of candida infections in surgical patients (and other high-risk patients), as well as the recognized shortcomings of current prophylactic and therapeutic regimens.

**REFERENCES**


10. Alani, E., Cao, L., and Kleckner, N. A method for gene disruption that allows repeated use of URA3 selection in the construc-