

Effect of *INT1* Gene on *Candida albicans* Murine Intestinal Colonization¹

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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 reintegrand (CAG5, *int1/int1* + *INT1*), and sacrificed 3 and 7 days later for quantitative analysis of cecal *C. albicans*.

Results. Following oral inoculation with 10^3 *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 pres-

ence 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. © 1999 Academic Press

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

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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-blaster" 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-blaster 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 adherence 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 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 *URA3* 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 10^3 *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 pretreated 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

TABLE 1
Characteristics of *Candida albicans* Strains Used in This Study [17]

| Strain and genotype | Phenotype on | | Adhesion to HeLa cells | Mouse virulence ^b |
|--|------------------|-------------------------------------|------------------------|------------------------------|
| | Milk-Tween agar | Spider medium ^a | | |
| CAF2 <i>INT1/INT1</i> (parent strain) | Extensive hyphae | Wrinkled colonies with fuzzy edges | Maximal | Maximal |
| CAG1 <i>INT1/int1</i> (heterozygous disruption) | Moderate hyphae | Wrinkled colonies with smooth edges | Intermediate | Intermediate |
| CAG3 <i>int1/int1</i> (homozygous disruption) | Few filaments | Smooth colonies with smooth edges | Minimal | Minimal |
| CAG5 <i>int1/int1 + INT1</i> (heterozygous reintegrant) | Moderate hyphae | Wrinkled colonies with smooth edges | Intermediate | Intermediate |

^a 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.

^b Mortality in intravenously inoculated mice.

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 of 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. Ceca were weighed, transferred to an anaerobic chamber (Forma Scientific, Marietta, OH), homogenized, serially diluted, plated on agar media, and incubated 24 to 48 h as described [26].

Agar media included MacConkey agar for selective isolation of gram-negative aerobic/facultative bacteria, colistin nalidixic acid agar for selective isolation of aerobic/facultative gram-positive bacteria and yeast, Wilkins–Chalgren agar supplemented with 50 µg/ml gentamicin for isolation of anaerobic species in the presence of relatively large numbers of aerobic/facultative bacteria, Sabouraud's dextrose agar supplemented with 100 µg/ml gentamicin (Sigma) and 25 µg/ml vancomycin (Eli Lilly and Co., Indianapolis, IN) for quantitation of yeast in the presence of relatively large numbers of cecal bacteria, synthetic minimal medium [27] agar without uracil for cultivation of yeast containing the *URA3* gene, and yeast peptone dextrose agar for isolation of both Ura⁺ and Ura⁻ yeast. Agar media for yeast cultivation were incubated at 30°C, and all other agar media were incubated at 35°C. All media were incubated aerobically except Wilkins–Chalgren agar, which was incubated anaerobically. Bacteria were identified by standard techniques [28], with gram-negative bacilli identified by the API 20E system (bioMérieux Vitek, Inc., Hazelwood, MO). Because each of the four *C. albicans* strains forms hyphae in serum [17], identification of *C. albicans* was confirmed by Gram stain, coupled with observation of characteristic hyphal formation following incubation of yeast in rabbit serum for 3 h at 37°C. 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 to confirm that the genotype of the recovered *C. albicans* corresponded to that inoculated.

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 artifactual movement of microbes during sectioning. Sections were observed for specific location and morphology of *C. albicans*.

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 detect-

TABLE 2

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

| <i>C. albicans</i> strain ^a | Oral antibiotic | Day after oral <i>C. albicans</i> | <i>C. albicans</i> ^b | Average \pm SE log microbes per gram of cecum | | |
|--|-----------------|-----------------------------------|---------------------------------|---|---------------|--------------------|
| | | | | Aerobic/facultative bacteria | | Anaerobic bacteria |
| | | | | Gram-negative | Gram-positive | |
| None | No | 3 | ND ^c | 4.3 \pm 0.4 | 6.1 \pm 0.1 | 9.2 \pm 0.2 |
| CAF2 | No | 3 | ND | 3.8 \pm 0.4 | 6.4 \pm 0.2 | 8.9 \pm 0.1 |
| CAG1 | No | 3 | ND | 4.5 \pm 0.3 | 6.1 \pm 0.1 | 9.1 \pm 0.1 |
| CAG3 | No | 3 | ND | 4.5 \pm 0.6 | 6.0 \pm 0.1 | 9.0 \pm 0.1 |
| CAG5 | No | 3 | ND | 4.2 \pm 0.5 | 6.3 \pm 0.1 | 9.1 \pm 0.1 |

^a Experiment represents four mice per treatment group in each of two experiments, for a total of eight mice per group.

^b Data represent *C. albicans* recovered on minimal medium agar without uracil.

^c None detected.

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 (10^3) 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*)

| <i>C. albicans</i> strain ^a | Oral antibiotic | Day after oral <i>C. albicans</i> | <i>C. albicans</i> ^b | Average \pm SE log microbes per gram of cecum | | |
|--|-----------------|-----------------------------------|---------------------------------|---|---------------|--------------------|
| | | | | Aerobic/facultative bacteria | | Anaerobic bacteria |
| | | | | Gram-negative | Gram-positive | |
| CAF2 | Yes | 3 | 5.4 \pm 0.4 | 3.2 \pm 0.1 | 3.8 \pm 0.3 | ND ^c |
| CAG1 | Yes | 3 | 3.9 \pm 0.3 ^d | 4.2 \pm 0.5 | 3.4 \pm 0.2 | ND |
| CAG3 | Yes | 3 | 3.8 \pm 0.3 ^d | 3.0 \pm 0 | 3.3 \pm 0.2 | ND |
| CAG5 | Yes | 3 | 4.2 \pm 0.4 ^e | 3.9 \pm 0.5 | 3.2 \pm 0.1 | ND |
| CAF2 | Yes | 7 | 5.9 \pm 0.4 | 5.6 \pm 0.5 | 3.1 \pm 0.1 | ND |
| CAG1 | Yes | 7 | 4.5 \pm 0.4 ^d | 6.3 \pm 0.5 | ND | ND |
| CAG3 | Yes | 7 | 4.1 \pm 0.4 ^d | 4.8 \pm 0.5 | ND | ND |
| CAG5 | Yes | 7 | 4.8 \pm 0.4 ^e | 6.4 \pm 0.6 | 3.2 \pm 0.1 | ND |

^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, $P = 0.07$.

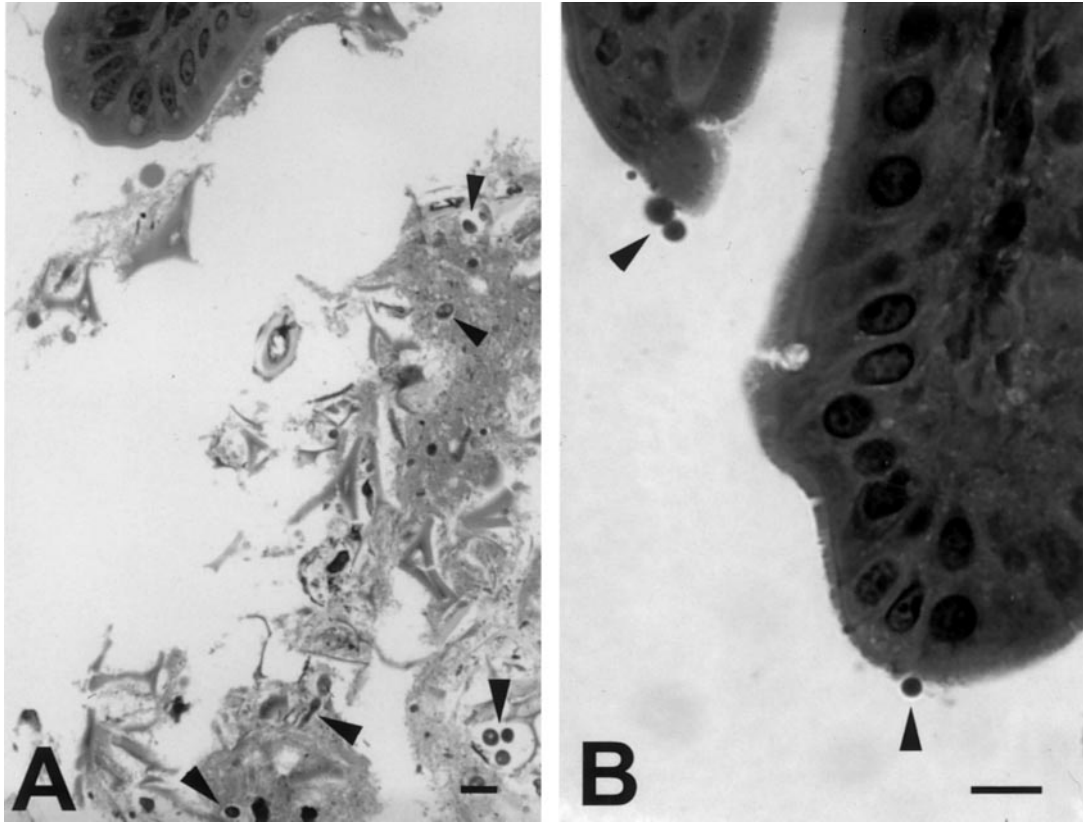


FIG. 1. Ileal sections from mice sacrificed 3 days after oral inoculation with *C. albicans* CAG5 showing that *C. albicans* did not readily adhere to epithelium but preferentially colonized luminal contents. (A) Yeast and filamentous forms (some highlighted by arrowheads) colonizing the ileal lumen, with the tip of an ileal villus evident at the upper right. (B) Rare observation of yeast forms (arrowheads) that appear adherent to ileal apical microvilli. Bars = 10 μ m.

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 \pm 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, 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 $10^{5.4}$ /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 10^3 or 10^7 *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.

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