Gastrointestinal Colonization by *Candida albicans* Mutant Strains in Antibiotic-Treated Mice

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Received 1 June 2000/Returned for modification 17 August 2000/Accepted 24 October 2000

Antibiotic-treated mice orally inoculated with one of three *Candida albicans* strains (including two mutant strains) or indigenous *Candida pelliculosa* showed levels of candidal gastrointestinal colonization that were strain specific. However, regardless of strain, the numbers of viable candida were intermediate to high in the stomach, were consistently low in the upper small intestine, and increased progressively down the intestinal tract.

In humans, *Candida albicans* is a member of the indigenous flora of the digestive tract, but it is also a potential pathogen and a frequent cause of complicating systemic infection in immunosuppressed patients, trauma patients, postsurgical patients, diabetics, premature infants, and patients infected with human immunodeficiency virus type 1 (5, 7, 20). Risk factors include neutropenia; use of vascular catheters, broad-spectrum antibiotics, and total parenteral nutrition; hemodialysis; oral mucosal colonization; abdominal surgery; prematurity; damage to the intestinal mucosa; burns; and chronic corticosteroid therapy (5, 20). *Candida* species number among the most common nosocomial pathogens in the United States, and *C. albicans* accounts for the majority of all fungal isolates (20). Increased intestinal colonization is generally accepted as a major risk factor that predisposes high-risk patients to systemic candidiasis (5).

The mouse has often been used in studies designed to clarify the pathogenesis of systemic candidiasis, making it important to understand the ability of *C. albicans* to colonize the mouse gastrointestinal (GI) tract. The concentration of intestinal *C. albicans* has often been correlated with the incidence of systemic infection, and several investigators (including ourselves) have assumed that the cecum is a representative site that can be used to monitor mouse GI colonization by *C. albicans* (2, 6, 12–16), although the reasons for this choice have largely been anecdotal.

Due to recent advances in genetic manipulation of *C. albicans*, it is now possible to sequentially disrupt both copies of a single gene in this diploid organism by use of the ura-blaster technique (1, 8, 11). This technique uses a *hisG*-URA3-*hisG* cassette to disrupt one copy of the gene in question, resulting in a heterozygous disruptant carrying the *URA3* marker. Spontaneous excision of *URA3* and recombination of the *hisG* repeats results in a heterozygous mutant lacking the *URA3* marker. A second round of disruption with this heterozygote results in a homozygous disruptant carrying the *URA3* marker in one allele. Mutant strains generated by this technique are being used in a variety of in vitro and in vivo studies designed to clarify the role(s) of specific genes in *C. albicans* pathogenesis. To our knowledge, there have been no studies that have clarified the comparative abilities of wild-type and mutant strains generated by the ura-blaster method to colonize the mouse GI tract. This may be important because strains with ura-blaster-mediated genetic disruptions likely have altered orotidine 5′-monophosphate decarboxylase enzyme activity (19). This enzyme is encoded by the *URA3* gene, and there is evidence that the activity of this enzyme may affect virulence in *C. albicans* (17, 19). Thus, *C. albicans* strains with altered orotidine 5′-monophosphate decarboxylase activity may have an altered ability to colonize the mouse GI tract. Furthermore, the additional genetic manipulations associated with gene disruption may also affect colonization.

Herein we compare the abilities of three exogenous strains of *C. albicans* and one indigenous *Candida* species, namely, *Candida pelliculosa*, to colonize the esophagus, stomach, upper and lower small intestine, cecum, and colon of antibiotic-treated mice. *Candida* species cannot be consistently recovered from the mouse intestinal flora, and *C. albicans* is not a member of the mouse normal flora (4, 21). *C. albicans* CAF2 and CAG3 were generated by the ura-blaster technique. *C. albicans* CAF2 (*int1/INT1 URA3/ura3::imm434*) was obtained from W. A. Fonzi, Georgetown University, Washington, D.C. (8). Construction of *C. albicans* CAG3 (*int1::hisG/int1::hisG-URA3-hisG ura3::imm434/ura3::imm434*) has been described previously (10), and characteristics of CAF2 and CAG3 have been published elsewhere (3, 9, 10). Compared to CAG3 (*int1/int1*), CAF2 (*int1/INT1*) demonstrates extensive hyphal development on agar media known to stimulate filamentation, shows increased adhesion to cultured epithelial cells (HeLa cells), and causes increased mortality in intravenously inoculated mice. The strain with the null mutation, CAG3 (*int1/int1*), has markedly reduced hyphal formation on agar media, shows minimal adherence to HeLa cells, and causes minimal mortality in intravenously inoculated mice. CAF2 has orotidine 5′-monophosphate decarboxylase activity typical of wild-type *C. albicans*, while CAG3 has markedly reduced activity (19). *C. albicans* 315 is a clinical isolate from the blood of a human, and this strain demonstrates filamentation typical of the species, i.e., germ tubes, pseudohyphae, and hyphae. *C. pelliculosa* M33

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is an indigenous yeast isolated from the cecum of the same mouse strain used in this study. *C. pelliculosa* is capable of forming pseudohyphae but is not capable of forming germ tubes or true hyphae. Stock cultures of all *Candida* strains were maintained at 280°C in Sabouraud's dextrose broth (Difco Laboratories, Detroit, Mich.) supplemented with 15% glycerol. For oral inoculation into mice, stock cultures were plated on minimal medium agar (9) supplemented with 2% dextrose, incubated at 30°C for 48 h, and then inoculated into minimal medium dextrose broth, incubated at 30°C with shaking for 18 h, washed, and resuspended in sterile saline. The yeast concentration was determined with a hemocytometer and was verified by quantitative culture on Sabouraud's dextrose agar incubated 48 h at 30°C. All strains grew exclusively as blastoconidia (budding yeast) under these conditions.

Six-week-old female Swiss Webster mice (weight, 18 to 22 g) were purchased from Harlan Sprague-Dawley, Indianapolis, Ind. Experiments were performed according to National Institutes of Health guidelines on the use of experimental animals, and all protocols were approved by the University of Minnesota Institutional Animal Care and Use Committee. Mice were pretreated for 3 days with drinking water containing 1 mg of bacitracin (Sigma Chemical Co., St. Louis, Mo.) per ml, 2 mg of streptomycin sulfate (Sigma) per ml, and 0.1 mg of gentamicin sulfate (Sigma) per ml and were then orally inoculated with a feeding needle (No. 9921; Popper & Sons, Inc., New Hyde Park, N.Y.) with 10^7 *C. albicans* 315, CAF2, or CAG3 or *C. pelliculosa* suspended in 0.1 ml of sterile saline. Antibiotic treatment was continued for the duration of the experiment. Mice were killed 3 days later. To eliminate cross-contamination among the various treatment groups, mice were housed in cages with filter tops and were handled by specially trained personnel. These conditions have been shown to eliminate

**TABLE 1.** Recovery of candida from esophageal tissue of antibiotic-treated mice orally inoculated with *C. albicans* 315, CAF2, or CAG3 or *C. pelliculosa*

<table>
<thead>
<tr>
<th>Candida species or strain</th>
<th>No. of mice with esophageal candida/total no. mice (%)</th>
<th>Avg ± SE no. of viable candida/esophagus*</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>C. albicans</em> 315</td>
<td>3/8 (38)</td>
<td>440 ± 266</td>
</tr>
<tr>
<td><em>C. albicans</em> CAF2</td>
<td>3/8 (38)</td>
<td>17 ± 7</td>
</tr>
<tr>
<td><em>C. albicans</em> CAG3</td>
<td>2/8 (25)</td>
<td>10 ± 0</td>
</tr>
<tr>
<td><em>C. pelliculosa</em></td>
<td>0/8 (0)</td>
<td>Not applicable</td>
</tr>
</tbody>
</table>

* Average for mice with detectable yeast.
cross-contamination of inoculated strains among mouse treatment groups (16).

Mice were killed by cervical dislocation, and their tissues were aseptically excised for quantitative analysis of aerobic and facultative microbial flora. Tissues included the esophagus (entire), stomach, upper small intestine (stomach to ligament of Treitz), lower small intestine (ligament of Treitz to ileal-cecal junction), cecum, and colon. The tissues were weighed, homogenized, serially diluted, plated on agar media, and incubated for 24 to 48 h as described previously (2, 16). Agar media included MacConkey agar for selective isolation of gram-negative aerobic bacteria and colistin-nalidixic acid agar for selective isolation of aerobic gram-positive bacteria and yeast. We have shown in previous work that yeast grow equally well on colistin-nalidixic acid agar and Sabouraud's dextrose agar (16), and use of both MacConkey and colistin-nalidixic acid agars provides optimum recovery of both bacteria and yeast (18). Microbes were identified by standard techniques (18).

Excluding the esophageal segment, microbes were enumerated as the log_{10} number of viable organism per gram (wet weight) of tissue with contents, and the lower limit of detection was 3.0 log_{10} per gram of tissue. Esophageal microbes were enumerated as the total number per tissue, and the lower detection limit was 10 microbes per tissue. For statistical analysis, values below the lower detection limit were assigned a value equal to the lower detection limit. Data were analyzed by analysis of variance followed by Fisher's test for significant difference. Statistical analyses were performed with StatView (version 5.0; Abacus Concepts, Berkeley, Calif.), and significance was set at a P value of <0.05. Similar data were pooled from each of two experiments with four mice per treatment group, for a total of eight mice per treatment group.

No microbial flora, other than the inoculated candidal strain, was detected in any tissue analyzed. C. albicans 315, CAF2, and CAG3 were recovered from esophageal tissues of some, but not all, mice, and C. pelliculosa was not detected in the esophagus (Table 1). Sporadic recovery from esophageal tissue suggested that this was not a site of active colonization, and the recovered candida likely reflected transient microbes ingested by these coprophagic animals.

Analysis of individual GI segments (Fig. 1A) indicated that colonization was similarly high for the wild-type strain C. albicans 315 and CAF2, the parent strain (INT1/INT1) used to produce the strain with the null mutation, strain CAG3 (INT1/INT1). Colonization was similar for C. albicans CAG3 and the indigenous C. pelliculosa strain, and both strains were recovered from GI segments in lower numbers compared to the numbers for C. albicans 315 and CAF2 (Fig. 1A). Analysis of individual candidal strains revealed a similar colonization pattern for each strain (Fig. 1B). The numbers of candida were consistently lowest in the upper small intestine and increased progressively down the intestinal tract, with the highest numbers recovered from the colon. Interestingly, the numbers of candida recovered from the stomach were typically similar to the relatively high numbers recovered from the lower small intestine or cecum (Fig. 1B). This pattern of intestinal colonization was similar to that reported for a mouse isolate of Torulopsis (Candida) pintolopesii (22).

To visualize in vivo morphology, cecal contents were analyzed from all mice. Cecal contents were rinsed from the tissue with a minimal volume (1 to 2 ml) of sterile saline, and a minimal volume (100 to 200 µl) was stained with calcofluor according to the manufacturer's directions (Fungi-Fluor kit; Polysciences, Inc., Warrington, Pa.) (Fig. 2). The remaining tissue and contents were used for quantitative culture as described above. Specimens were examined under an epifluorescence microscope. Fungal elements (100 from each of eight mice per treatment group) were identified as either yeast or filamentous forms, the latter defined as an outgrowth four times the width of the mother cell or a chain of four or more elongated yeast cells. The C. pelliculosa and C. albicans CAG3, CAF2, and 315 strains showed 8.3% ± 3.7%, 3.7% ± 1.5%, 14.3% ± 4.3%, and 40.0% ± 15.4% (average ± standard error) filamentation, respectively. Filamentation of C. albicans 315 was increased compared to that for each of the other strains (P < 0.01 by analysis of variance with Fisher's post hoc test). Phillips and Balish (21) reported that C. albicans was 100% yeast forms in stomach contents from conventional mice but 40% filamentous forms in stomach contents from germfree mice that had been monoassociated with C. albicans. In the present study, the morphology of cecal C. albicans appeared to be more similar to that reported for monoassociated mice than for conventionally reared mice. This seemed reasonable because, in the present study, none of the mice had detectable competing aerobic or facultative bacterial flora, and the inoculated candidal strain was the only microbe recovered from the GI tract.

In summary, although some candidal strains colonized the mouse GI tract in greater or lesser numbers, the pattern of
colonization was similar from strain to strain. This consistency included exogenous C. albicans and indigenous C. pelliculosa, as well as C. albicans subjected to manipulations associated with the ura-blaster technique of mutagenesis. Either the stomach, lower small intestine, cecum, or colon could be used to monitor and/or compare colonization by different candidal strains, including strains with altered orotidine 5'-monophosphate decarboxylase activity associated with ura-blaster mutagenesis.

This work was supported by Public Health Service grant GM59221 from the National Institutes of Health, as well as grant 9906100 from the March of Dimes Birth Defects Foundation.

REFERENCES