Ecology of Candida albicans Gut Colonization: Inhibition of Candida Adhesion, Colonization, and Dissemination from the Gastrointestinal Tract by Bacterial Antagonism

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Received 19 December 1984/Accepted 20 May 1985

Antibiotic-treated and untreated Syrian hamsters were inoculated intragastrically with Candida albicans to determine whether C. albicans could opportunistically colonize the gastrointestinal tract and disseminate to visceral organs. Antibiotic treatment decreased the total population levels of the indigenous bacterial flora and predisposed hamsters to gastrointestinal overgrowth and subsequent systemic dissemination by C. albicans in 86% of the animals. Both control hamsters not given antibiotics and antibiotic-treated animals reconstituted with an indigenous microflora showed significantly lower gut populations of C. albicans, and C. albicans organisms were cultured from the visceral organs of 0 and 10% of the animals, respectively. Conversely, non-antibiotic-treated hamsters inoculated repeatedly with C. albicans had high numbers of C. albicans in the gut, and viable C. albicans was recovered from the visceral organs of 53% of the animals. Examination of the mucosal surfaces from test and control animals indicated further that animals which contained a complex indigenous microflora had significantly lower numbers of C. albicans associated with their gut walls than did antibiotic-treated animals. The ability of C. albicans to associate with intestinal mucosal surfaces also was tested by an in vitro adhesion assay. The results indicate that the indigenous microflora reduced the mucosal association of C. albicans by forming a dense layer of bacteria in the mucus gel, out-competing yeast cells for adhesion sites, and producing inhibitor substances (possibly volatile fatty acids, secondary bile acids, or both) that reduced C. albicans adhesion. It is suggested, therefore, that the indigenous intestinal microflora suppresses C. albicans colonization and dissemination from the gut by inhibiting Candida-mucosal association and reducing C. albicans population levels in the gut.

Systemic Candida infections are important causes of morbidity and mortality among patients who are compromised immunologically or who are undergoing protracted antibiotic therapy (41, 52, 69). The passage of viable Candida albicans through the gastrointestinal (GI) mucosa into the host bloodstream is believed to be an important mechanism leading to systemic candidosis (38, 61, 62), particularly in patients with acute leukemia (43). Patient and animal studies support this hypothesis and indicate that a number of opportunistic fungal pathogens can spread systemically from the intestinal lumen to invade visceral organs (28, 29, 67, 68). We recently reported, for instance, that C. albicans, Candida parapsilosis, Candida pseudotropicalis, Candida tropicalis, and Torulopsis glabrata could opportunistically colonize the gut and disseminate to visceral organs after intragastric challenge of antibiotic-treated mice (37). The feeding of C. albicans to animals not given antibiotics, in contrast, does not lead to Candida dissemination from the GI tract (13, 37, 67) unless extremely high numbers of yeasts are ingested (38, 61, 62), suggesting that large numbers of fungi may be a determinant of fungal dissemination (45).

Since antibiotic treatment predisposes animals to GI overgrowth and subsequent dissemination by Candida organisms, it has been suggested that members of the indigenous intestinal microflora suppress the growth of C. albicans within the gut, thereby preventing systemic invasion (43, 61, 62). Several studies have shown that certain intestinal bacteria are inhibitory to both in vitro growth and GI colonization by C. albicans (4, 32-34, 44, 47, 48). It is important to note, however, that these studies do not reflect interactions as they normally occur in the intestinal tract (17, 21). Most, if not all, in vitro studies have reported on C. albicans suppression by a single bacterial species (45), which is hardly representative of the 400 to 500 different bacterial species that normally inhabit the intestinal tract (54). Likewise, studies in which a monoflora of Escherichia coli (or any other single bacterial species) antagonized C. albicans growth in the gut of gnotobiotic animals (4, 32, 33, 44) cannot be expected to reflect interactions of a complex indigenous microflora (17, 21). Bacteria under the former condition reach abnormally high numbers in the gut (31). E. coli itself is suppressed by the strict anaerobes which dominate the intestinal microflora (22, 63). Thus, it is still not known whether E. coli can exert an inhibitory mechanism(s) over C. albicans under normal in vivo conditions, as has so often been suggested (4, 32-34, 44, 45). In fact, Clark (10) showed that C. albicans grew unchecked for several weeks in the GI tracts of gnotobiotic mice containing an intestinal flora of Bacteroides sp., Lactobacillus sp., Streptococcus faecalis, Streptococcus lactis, and E. coli. Recent studies also indicate that enteric bacilli (including E. coli) do not inhibit the growth of C. albicans in the GI tracts of conventional mice (2, 37a). The present studies were initiated to examine the mechanisms by which a complex indigenous microflora inhibits C. albicans colonization and dissemination from the GI tract.
To test the ability of indigenous cecal bacteria to protect against _C. albicans_ colonization and dissemination, cecal homogenates (or filtrates as controls) were administered to antibiotic-decontaminated hamsters (by both oral and rectal routes) once daily for 5 consecutive days, beginning 5 days after stopping antibiotic treatment. For oral administration, hamsters were lightly anesthetized with methoxyflurane (Pittman-Moore Co., Washington Crossing, N.J.) and cecal homogenates or filtrates administered by carefully inserting a 5-cm 18-gauge plastic catheter through the mouth into the stomach and injecting 1 ml of the material. For rectal administration, the same catheter was placed approximately 5 mm into the rectum, the skin was pinched tightly around the catheter, and 2 ml of the material was injected. The latter dose has been shown to be an amount adequate to reach the cecum (66).

On the day after the last administration of cecal homogenates, or filtrates, the animals were challenged intragastrically (as above) with 0.5 ml of Sabouraud dextrose broth containing 10^4 CFU of _C. albicans_. Another group of hamsters not treated with antibiotics also was inoculated with 10^4 CFU of _C. albicans_ intragastrically at 0, 8, and 16 h after the first yeast challenge, to maintain high numbers of _C. albicans_ organisms in the gut without antibiotic treatment.

**Enumeration of _C. albicans_ in tissues.** Counts of viable _C. albicans_ were performed on cecal contents and visceral organs 24 h after administration of _C. albicans_. Animals receiving multiple inoculations of _C. albicans_ were cultured for _C. albicans_ 24 h after the first yeast challenge. Hamsters were sacrificed by cervical dislocation, their abdomens were opened, and the contents of the cecum and small intestine were collected into sterile tubes. Cecal homogenates were administered to hamsters within 15 min of preparation. Filtrates of cecal contents were prepared by a modification of a previously described method (53). Briefly, cecal homogenates (prepared as described above) were removed from the anaerobic chamber and centrifuged twice at 10,000 x g for 15 min. The remaining supernatant then was passed through 0.20-µm filters (Millipore Corp., Bedford, Mass.) to remove any remaining bacteria and placed in 10-ml glass ampoules for transport to a separate group of antibiotic-treated hamsters. Cecal homogenates were administered to hamsters within 15 min of preparation.

**Enumeration of indigenous cecal bacteria.** Cecal population levels of indigenous bacteria were determined in antibiotic-treated and control animals in the anaerobic chamber. The ceca were removed aseptically and homogenized in a Waring blender vessel containing 30 ml of sterile, pre-reduced tryptic soy broth (Difco) with 0.04% (wt/vol) NaCl to compensate for 5% CO_2_ in the anaerobic chamber (65). These cecal homogenates were placed into 50-ml sterile tubes, mixed vigorously, and allowed to settle briefly. The resulting supernatants containing the indigenous cecal microflora were transferred inside the anaerobic chamber to 10-ml glass ampoules, stoppered, and placed on ice for transport to rigid plastic germfree isolators (Germfree Laboratories, Inc., Miami, Fla.) containing antibiotic-treated hamsters. Cecal homogenates were administered to hamsters within 15 min of preparation.

**Fungi.** A clinical isolate of _C. albicans_ (CA34) recovered from the blood of a patient with systemic candidosis was maintained on Sabouraud dextrose agar (Difco Laboratories, Detroit, Mich.) slants at 22 to 25°C. For intragastric colonization and dissemination, cecal homogenates (or filtrates as controls) were administered to hamsters within 15 min of preparation.
TABLE 1. Association of C. albicans with intestinal slices

<table>
<thead>
<tr>
<th>System</th>
<th>Assay</th>
<th>Source of intestinal slices*</th>
<th>Log, mean no. of C. albicans/slice</th>
<th>Association index</th>
<th>Log, mean no. of C. albicans/slice</th>
<th>Association index</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>IC</td>
<td>Antibiotic-treated hamsters</td>
<td>2.4</td>
<td>0.13</td>
<td>2.5</td>
<td>0.08</td>
</tr>
<tr>
<td>2</td>
<td>IF</td>
<td>Antibiotic-treated hamsters</td>
<td>4.2</td>
<td>2.62</td>
<td>4.0</td>
<td>1.82</td>
</tr>
<tr>
<td>3</td>
<td>PBS</td>
<td>Antibiotic-treated hamsters</td>
<td>5.0</td>
<td>13.41</td>
<td>4.8</td>
<td>13.04</td>
</tr>
<tr>
<td>4</td>
<td>IC</td>
<td>Untreated hamsters</td>
<td>2.5</td>
<td>0.09</td>
<td>2.4</td>
<td>0.06</td>
</tr>
<tr>
<td>5</td>
<td>IF</td>
<td>Untreated hamsters</td>
<td>3.8</td>
<td>0.60</td>
<td>3.4</td>
<td>0.25</td>
</tr>
<tr>
<td>6</td>
<td>PBS</td>
<td>Untreated hamsters</td>
<td>3.9</td>
<td>0.70</td>
<td>3.7</td>
<td>0.73</td>
</tr>
<tr>
<td>7</td>
<td>PBS + BA</td>
<td>Antibiotic-treated hamsters</td>
<td>NT</td>
<td>NT</td>
<td>4.3</td>
<td>4.19</td>
</tr>
<tr>
<td>8</td>
<td>PBS + VFA</td>
<td>Antibiotic-treated hamsters</td>
<td>4.1</td>
<td>1.17</td>
<td>4.1</td>
<td>1.86</td>
</tr>
</tbody>
</table>

* PBS + BA, PBS containing bile acids (lithocholic acid, 3.0 mM; deoxycholic acid, 2.6 mM); PBS + VFA, PBS containing VFA (valeric acid, 1.2 mM; isovaleric acid, 2.2 mM; butyric acid, 12.4 mM; isobutyric acid, 1.4 mM; propionic acid, 20.1 mM; acetic acid, 49.3 mM).

Antibiotic-treated hamsters given VAG for 3 days as described in the text.

NT, Not tested.

The association index is described in the text.

To test the ability of C. albicans to associate with intestinal mucosal surfaces in vivo, untreated and antibiotic-treated (given penicillin G [500 U/ml] for 3 days as described above) animals were injected with 0.5 ml of Sabouraud dextrose broth containing 10⁷ CFU of C. albicans. Twenty-four hours later, counts of the numbers of viable C. albicans were determined for cecal contents, cecal walls, and visceral organs. In addition, counts of the numbers of indigenous anaerobic bacteria and enteric bacilli per cecal contents or per gram of cecal wall were determined by plating 0.1 ml from serial 10-fold dilutions on All agar and Tergitol-7 agar (Difco; 7) as described above. Several animals from each group also were sacrificed, and their intestinal tissues were examined by scanning electron microscopy (SEM) as described below.

The rate of C. albicans disassociation from intestinal tissues also was measured from ceca of antibiotic-treated and untreated animals challenged with C. albicans. Ceca were removed aseptically and opened, and nonadherent fungi and digesta were removed by washing with sterile PBS. Cecal tissues then were placed in 10 ml of sterile PBS and incubated on a Gyrotory shaker (at 10 rpm; New-Brunswick Scientific Co., Inc., Edison, N.J.) at 37°C. The amount of viable C. albicans cells in the surrounding PBS solution was determined by plating 0.1 ml from serial 10-fold dilutions at various intervals.

Variation in mucosal association assay parameters. C. albicans adhesion to intestinal mucosal surfaces was examined by performing the above adhesion assay in various test solutions. Solutions included intestinal slices from untreated or antibiotic-treated hamsters (Table 1). Antibiotic-treated animals were sacrificed and placed directly in an anaerobic chamber. Small bowel and cecum then were removed aseptically, and their contents were emptied into separate sterile test tubes, which were brought to 10 ml with PBS and mixed vigorously for 20 s. Indigenous microbes were removed from similar preparations by filtration (X2) through 0.20-μm Millipore filters and termed intestinal filtrates (IF). PBS alone, or mixed with secondary bile acids or volatile fatty acids (VFA), was used as the assay solution (Table 1).

SEM. Intestinal tissues were fixed overnight at 4°C by immersion in 2.5% gluteraldehyde in 0.2 M cacodylate buffer (pH 7.4), then washed in two changes of buffer, and postfixed in 1% osmium tetroxide in the same buffer for 1 h. The tissue then was washed in distilled water, dehydrated in acidified 2.2-dimethoxypropane (42), infiltrated with absolute alcohol, and critical point dried with a Polaron E5100 critical-point dryer. Specimens were coated under vacuum with gold-palladium (60:40) in a Polaron sputter coater equipped with an omnirotary stage and examined in an ISI Super II scanning electron microscope at 15 kV.

Cecal VFA. The concentrations of cecal VFA were determined chromatographically (11; Supelco, Inc., Bellefonte, Pa., GC separation of VFA C2-C5, bulletin no. 749D, 1975) by a modification of an internal standard (2-methylpentanoic acid (2-MPMA) method) (40). The cecal contents of sacrificed antibiotic-treated and control animals were expelled into sterile preweighed tubes and mixed with 1 ml of 25% (v/vol) metaphosphoric acid (Sigma Chemical Co., St. Louis, Mo.) and 1 ml of 2-methylpentanoic acid (11.2 mM Pfaltz and Bauer, Stamford, Conn.) for every 2 g of cecal content. The samples then were stoppered, mixed, and held at −70°C overnight. Samples were brought to room temperature, mixed, and centrifuged for 15 min at 17,500 x g at 10°C. After centrifugation, the supernatants were filtered through 0.20-µm filters, dispersed into 1.0-ml screw-capped autosampler vials (Phase Separation, Inc., Norwalk, Conn.), and loaded into a Varian model 8000 autosampler. The following standard VFA mixture also was used for comparison and calculation of the unknowns: acetic acid (Malinckrodt, Inc., St. Louis, Mo.; 91.0 mM) propionic acid (Aldrich Chemical Co., Inc., Milwaukee, Wis.; 35.1 mM); and butyric, isobutyric, valeric, and isovaleric acids (Eastman Chemical Products, Inc., Kingsport, Tenn.; 16.97, 2.18, 2.03, and 1.80 mM, respectively). A Varian model 6000 gas chromatograph equipped with a flame ionization detector was used. The chromatograph and autosampler were controlled by a Varian 401 chromatography data system.
TABLE 2. Population levels of indigenous bacteria in the ceca of antibiotic-treated and control animals

<table>
<thead>
<tr>
<th>Animal treatment</th>
<th>Facultative bacteria</th>
<th>Strictly anaerobic bacteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>Antibiotics</td>
<td>2.4 ± 0.8</td>
<td>&lt;2.4</td>
</tr>
<tr>
<td>Antibiotics, followed by cecal filtrates</td>
<td>2.4 ± 0.7</td>
<td>&lt;2.4</td>
</tr>
<tr>
<td>Antibiotics, followed by cecal homogenates</td>
<td>6.0 ± 0.8</td>
<td>8.8 ± 0.4</td>
</tr>
<tr>
<td>None</td>
<td>6.3 ± 0.7</td>
<td>8.9 ± 0.2</td>
</tr>
</tbody>
</table>

Values expressed are from three animals per group.

**RESULTS**

Indigenous bacterial populations and cecal characteristics.

The VAG treatment of hamsters used in this study reduced the total population size of the indigenous bacterial flora more than a millionfold (Table 2; \( P < 0.001 \)). Furthermore, aerobic and anaerobic cultures of IC from these animals revealed that the strictly anaerobic flora was effectively eliminated, whereas the facultative flora contained primarily enterococci. Gram stains also showed antibiotic-treated animals to contain few bacteria (mostly gram-positive cocci), with a complete disappearance of the predominant gram-negative and fusiform-shaped rods. In contrast, antibiotic-treated hamsters which had been reconstituted with cecal bacteria by oral and rectal injections of cecal contents had numbers of facultative and anaerobic bacteria colonizing their intestinal tracts that were similar to those found in conventional animals (Table 2). Gram stains of IC from reconstituted hamsters showed a bacterial flora resembling that of untreated animals. *C. albicans*, or other *Candida* spp., were not found to be commensals of the GI tracts of the hamsters used in the present study.

Dissection of test and control hamsters revealed several different cecal characteristics according to the experimental group examined. Animals that possessed a complex bacterial flora had small ceca (approximately 1 to 2% of their total body weight), which contained thick, pasty contents. Hamsters given antibiotics to eliminate the indigenous microflora, in contrast, had enlarged ceca (5 to 10% of their total body weight) with very watery contents, a trait attributable to mice lacking a complex intestinal microflora (33, 55).

The concentrations of most of the individual and the total VFA in the cecal contents of antibiotic-treated hamsters were usually less than half the amounts found in control animals (Table 3). Isovalerate concentrations, however, were slightly higher in antibiotic-treated animals, and isobutyrate concentrations in the cecal contents of VAG-treated animals were ca. 3.5 times those of the control animals (Table 3).

**C. albicans colonization and dissemination from the GI tract.** The ability of *C. albicans* to colonize and disperse from the GI tracts of test and control hamsters is summarized in Table 4. Antibiotic-treated animals challenged with *C. albicans* had high cecal numbers of *C. albicans* colonizing the gut 24 h after oral inoculation, and viable *C. albicans* recovered from the visceral organs of 86% of the animals. Similarly, antibiotic-treated hamsters given cecal filtrates before yeast challenge had large numbers of *C. albicans* present in the GI tract, and viable *C. albicans* was recovered from the visceral organs of 85% of the animals. Control animals not receiving antibiotic therapy, and antibiotic-treated hamsters reconstituted with the indigenous intestinal flora, in contrast, had significantly lower cecal populations of *C. albicans* (\( P < 0.001 \)). *C. albicans* organisms spread systemically from the gut to visceral organs in 0 and 10% of these animals, respectively, and the incidence of systemic spread to these organs was significantly lower (\( P < 0.001 \)) than that in hamsters lacking a complex intestinal microflora.

These data supported the hypothesis that threshold population levels of *C. albicans* are an important determinant of fungal dissemination (45). To test this hypothesis further, an untreated group of hamsters was inoculated intragastrically.

TABLE 3. Concentration of VFA in the ceca of antibiotic-treated and control hamsters

<table>
<thead>
<tr>
<th>Animal treatment</th>
<th>Acetic (mM)</th>
<th>Propionic (mM)</th>
<th>Isobutyric (mM)</th>
<th>Butyric (mM)</th>
<th>Isovaleric (mM)</th>
<th>Valeric (mM)</th>
<th>Total (mM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>None (control)</td>
<td>118.6 ± 19.9</td>
<td>20.4 ± 4.5</td>
<td>4.4 ± 0.8</td>
<td>20.7 ± 6.3</td>
<td>3.6 ± 0.4</td>
<td>1.3 ± 0.1</td>
<td>168.2 ± 31.3</td>
</tr>
<tr>
<td>Penicillin</td>
<td>29.1 ± 3.9</td>
<td>20.4 ± 3.9</td>
<td>2.7 ± 1.0</td>
<td>2.7 ± 0.7</td>
<td>3.9 ± 0.7</td>
<td>0.5 ± 0.1</td>
<td>45.6 ± 4.9</td>
</tr>
<tr>
<td>VAG</td>
<td>25.8 ± 2.9</td>
<td>21.8</td>
<td>3.3 ± 0.4</td>
<td>3.3 ± 2.1</td>
<td>4.0 ± 0.5</td>
<td>0.7 ± 0.6</td>
<td>53.4 ± 5.0</td>
</tr>
</tbody>
</table>

Animals were given nothing, penicillin, or VAG ad libitum in the drinking water as described in the text.

Values are mean ± standard deviation of five animals per group. Values within parentheses are percents of control group.
TABLE 4. Incidence of dissemination and organ population levels of C. albicans in antibiotic-treated and control hamsters

<table>
<thead>
<tr>
<th>Animal treatment</th>
<th>Incidence of dissemination</th>
<th>Log., mean CFU of C. albicans* in:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Cecum</td>
</tr>
<tr>
<td>Antibiotics</td>
<td>13/15</td>
<td>7.7 ± 0.7</td>
</tr>
<tr>
<td>Antibiotics, followed by cecal filtrates</td>
<td>17/20</td>
<td>7.6 ± 0.6</td>
</tr>
<tr>
<td>Antibiotics, followed by cecal homogenates</td>
<td>2/20</td>
<td>3.8 ± 0.7</td>
</tr>
<tr>
<td>None</td>
<td>0/20</td>
<td>3.4 ± 0.8</td>
</tr>
<tr>
<td>None (multiple C. albicans inoculations)</td>
<td>8/15</td>
<td>6.2 ± 0.6</td>
</tr>
</tbody>
</table>

* Antibiotic-treated animals received 3 days of VAG treatment as described in Table 2, footnote b.

TABLE 5. Populations of indigenous bacteria and C. albicans in the cecal contents, cecal walls, and visceral organs of untreated and penicillin-treated animals challenged with 10^6 C. albicans

<table>
<thead>
<tr>
<th>Animal treatment</th>
<th>Cecal contents (per g [wet wt])</th>
<th>Cecal wall (per g [wet wt])</th>
<th>Visceral organs</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Enteric bacilli</td>
<td>Anaerobes</td>
<td>C. albicans</td>
</tr>
<tr>
<td>None</td>
<td>5.2 ± 0.5</td>
<td>9.3 ± 0.5</td>
<td>3.3 ± 0.3</td>
</tr>
<tr>
<td>Penicillin</td>
<td>9.6 ± 0.3</td>
<td>7.4 ± 0.7</td>
<td>7.4 ± 0.2</td>
</tr>
</tbody>
</table>

* ND, Not detectable.

** Notice: the text contains numerical data and statistical measures, which are typical for scientific reports. The tables summarize the incidence of dissemination and organ population levels of C. albicans in antibiotic-treated and control hamsters, showing the effect of antibiotic treatment on the incidence of dissemination and the mean CFU of C. albicans in various organs. The data are presented in a structured format, facilitating easy comparison and analysis. The tables are essential for understanding the impact of antibiotic treatment on the mucosal microflora and the ability of C. albicans to associate with intestinal mucosal surfaces. The text also discusses the role of mucosal-associated microflora and the effect of antibiotics on the mucosal microflora.**
given antibiotics, yeast cells were probably associated with the loose top layer of mucus not preserved by the fixation procedure used for SEM studies. That is, most of the yeast cells found in untreated animals were probably associated with the thick layer of mucus gel covering the epithelium, whereas Candida cells were found throughout the cecal tissue of antibiotic-treated animals.

The rate of C. albicans disassociation from intestinal tissues from penicillin-treated and untreated animals (see above) also was tested. It was found that C. albicans cells were shed from intestinal mucosal surfaces at a faster rate from untreated animals than from antibiotic-treated animals.

Nearly 74% (range, 58.7 to 76.2%) of the Candida cells that had associated with intestinal mucosal surfaces of untreated animals (n = 15) were removed by 1 h of incubation, whereas only a mean 31.3% (range, 1.8 to 60.9%) of the Candida cells were removed from animals treated with antibiotics (n = 15). It should be noted that it is not known whether the in vitro results of the present study adequately reflect the disassociation rates of C. albicans in vivo, although it is likely that C. albicans disassociation from intestinal tissues would be higher from animals that possessed an indigenous microflora since C. albicans cells would not be able to penetrate to the same degree into the mucus gel.
FIG. 2. SEM of the cecum from a penicillin-treated animal challenged with C. albicans. Yeast cells can be seen attached to and embedded (arrow) in mucus material. Magnification, x7,000.

DISCUSSION

The results presented in this paper strongly support the hypothesis that the indigenous intestinal microflora suppresses C. albicans numbers in the GI tract and reduces the incidence of dissemination from the intestinal lumen to visceral organs (37, 37a, 43, 61, 62). The administration of normal cecal contents to antibiotic-treated animals before oral C. albicans challenge, for instance, had high numbers of C. albicans organisms forming thick layers of bacteria in the mucus gel (14) covering the epithelium. Hamsters treated with antibiotics, for instance, had high numbers of C. albicans organisms opportunistically colonizing their GI tracts, with a high incidence of dissemination to visceral organs. Animals that possessed an indigenous intestinal microflora, in contrast, had low numbers of C. albicans residing in their GI tracts, with only 2 of 40 animals showing signs of Candida dissemination. Of untreated hamsters that received multiple Candida challenges to maintain high cecal populations, however, 53% had viable C. albicans recovered from their visceral organs. Thus, the suppression of Candida population levels appears to be an important factor controlling fungal dissemination from the GI tract (15, 37, 37a). Similarly, it has been shown that high intestinal populations of bacteria were required to promote E. coli dissemination (translocation) from the GI tract (8). When intestinal population levels dropped below the threshold level due to antagonism by the indigenous intestinal microflora, E. coli cells could no longer disseminate from the gut (6, 9). Likewise, work in our laboratory previously has shown that certain fungi unable to maintain high population levels in the intestinal tracts of antibiotic-treated mice could not disseminate to visceral organs, although viable fungi remained in the gut for several days (36, 37). Other investigators also have suggested a direct relationship between GI populations of other microbes and systemic dissemination (60, 64).

Nevertheless, it also appears that the dense bacterial layers lining the mucosal epithelium may have provided an important defense mechanism that inhibited both Candida colonization and dissemination from the GI tract. It is apparent that the first step in mucosal association must be the penetration of the mucus gel (18, 20). In infant mice, which lack a complete bacterial flora including the dense microbial populations in the mucus gel (12, 56), C. albicans can readily associate with and pass through the gut wall to initiate systemic infection (15, 50). In the present study, in contrast, it was shown that intestinal tissues that possessed an indigenous wall-associated microflora strongly inhibited mucosal association and dissemination of C. albicans from the GI tract. Mucosal association of C. albicans appeared to be blocked by competing for adhesion sites and physically blocking the larger yeast cells from penetrating into the mucus gel. For example, when intestinal slices from antibiotic-treated hamsters, yeast cells, and intestinal bacteria were mixed at the same time (assay 1), mucosal association by C. albicans was as strongly inhibited as when the mucosal association assay was performed with IC and slices from untreated hamsters (assay 4). Although this may seem surprising at first, it should be noted that most indigenous mucosal bacteria are very motile (58, 59) and may have been chemotactically attracted to the mucosal surface very rap-
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60. van der Waaij, D., J. M. Berghuis-deVries, and J. E. C.