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A Model of Sustained Gastrointestinal Colonization by *Candida albicans* in Healthy Adult Mice

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Three-month-old male Crl:CD1(ICR)BR and C3H/HeJ mice were fed chow containing *Candida albicans* for 14 days, while similar control mice were fed regular food. Stool cultures were done for all mice before and after administration of the special diet. Stool cultures were repeated 48 h, 1 week, and 1 and 3 months after stopping the diet for Crl:CD1(ICR)BR mice and again 5 months afterward for C3H/HeJ mice. Some animals were sacrificed at the end of the special diet, and cultures and histopathologic examination of various organs were performed. Colonization with *C. albicans* occurred in the *Candida*-fed mice, and the fungus was maintained in the gastrointestinal tract at a concentration of 10^3 to 10^4 CFU/g of stool for up to 5 months. There was no histologic evidence of organ infection with *Candida* spp. The fungus was not found in stool cultures or organs of mice in the control group. The results suggest that persistent gastrointestinal colonization of adult mice by *C. albicans* can be achieved without immunosuppression. Thus, with additional manipulations, this model could be useful for studying the role of gastrointestinal colonization by *C. albicans* in the development of systemic infection.

Disseminated candidiasis is an increasing problem in immunocompromised patients (3, 19, 25). The source of dissemination in most of these patients is either an indwelling intravenous catheter or the gastrointestinal tract (3, 5, 7, 8, 15, 19). Experimental and clinical data suggest that *Candida* organisms can pass into the bloodstream by persorption through the intact gastrointestinal mucosa and spread to visceral organs, leading to systemic candidiasis (5, 13, 14, 16, 24, 25). This risk is increased by the widespread use of antibiotics that alter the microbial ecology and by antineoplastic agents that compromise host defenses and damage the mucosa (3, 5).

Many mouse models have been used for the study of disseminated candidiasis. Usually the mouse is infected by intravenous injection of the fungal organisms (1, 2, 10, 17, 18, 27). Gastrointestinal colonization by the fungus has also been attempted, but the healthy adult mouse has been resistant to sustained and high-grade gastrointestinal colonization by *Candida albicans* (8, 11, 28). Thus, previous candidiasis models used infant mice or antibiotic-treated and/or immunocompromised adult mice (7–9, 11–13, 15, 20, 22, 23, 28, 29).

In this study, we present a method of maintaining persistent high-grade gastrointestinal colonization by *C. albicans* in healthy untreated adult mice. This model may more closely mimic the events that occur in human adults with disseminated candidiasis and could be useful for studying the role of *Candida* colonization and subsequent dissemination from the gastrointestinal tract.

MATERIALS AND METHODS

Animals. Two mouse species were studied: male Crl:CD1(ICR)BR mice, 3 months old and weighing approximately 30 g each (Charles River Laboratories, Wilmington, Mass.), were used to establish the colonization model with three different inocula; and male C3H/HeJ mice of the same age and weighing approximately 25 g each (Jackson Laboratories, Bar Harbor, Maine) were colonized with only the optimal candidal inoculum to confirm that the results obtained with Crl:CD1(ICR)BR mice were not species related. Mice were separated into groups of 20 per inoculum and housed 5 mice per cage in cages with wire mesh bottoms to prevent coprophagia. Autoclavable food (Wayne Sterilizable Lab Blox, Allied Mills, Inc., Chicago, Ill.) and tap water were allowed *ad libitum*. Food and water were cultured on Sabouraud dextrose agar (SDA; BBL Microbiology Systems, Cockeysville, Md.) to exclude contamination with *C. albicans*.

Preparation of food containing *C. albicans*. A human pathogenic blood isolate of *C. albicans* shown to cause disseminated candidiasis when injected intravenously into Crl:CD1(ICR)BR mice was used for the preparation of the food containing *C. albicans* (17). Stock cultures were prepared from the original blood isolate and were stored in 2% low fat milk at −70°C. Cultures were then maintained at room temperature on SDA slants (Regional Media Laboratories, Lenexa, Kans.) subcultured once a month (37°C for 24 h.) The organism was then subcultured on fresh SDA plates (Regional Media Laboratories) for each experiment. A single colony from a 48-h growth on SDA was inoculated into a flask containing 6 ml of Sabouraud dextrose broth (Difco Laboratories, Detroit, Mich.) and was incubated at 37°C for 24 h. Continuous 24-h agitation of the flask (180 strokes per min) was performed in a subset of experiments on a shaker (Eberbach Corp., Ann Arbor, Mich.)

All animal food was ground to powder, divided into aliquots of 300 g, and sterilized by autoclaving (15 min at 121°C and 15-lbs/in^2 pressure).

We conducted three sets of experiments, using different candidal inocula to optimize the inoculum necessary for colonization: (i) a "high" inoculum of *C. albicans* in food resulted in gastrointestinal colonization with dissemination to organs, (ii) a "low" inoculum did not cause gastrointestinal colonization, and (iii) an intermediate or "optimal" inoculum was associated with gastrointestinal colonization without dissemination.

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The food was prepared as follows. (i) For preparation of the high inoculum, 6 ml of the overnight *Candida* suspension, which was agitated for 24 h on the shaker, was suspended into 360 ml of sterile water and subsequently mixed with 300 g of food powder. The median concentration of *C. albicans* in the suspension was 6.5×10^7 CFU/ml (mean, 6.6×10^7; range, 5.0×10^7 to 8.3×10^7 CFU/ml). The mixture was spread into 0.5-cm-thick layers on sterile plastic petri dishes (15 by 100 mm) and dried at 37°C for 48 h.

(ii) For preparation of the low inoculum, 6 ml of the overnight *Candida* suspension (without agitation) was suspended in 360 ml of sterile water and mixed with 300 g of food powder. The median concentration of *C. albicans* in the suspension was 5.7×10^6 CFU/ml (mean, 5.8×10^6; range, 2.7×10^6 to 9.0×10^6 CFU/ml). However, a different drying procedure was utilized: 20-ml beakers were filled with the mixture and dried for 48 h in a lyophilizer (Freeze-drier; VirTis Co., Gardiner, N.Y.). Altering the drying procedure was sufficient to alter the total number of viable colonies per gram of chow significantly.

(iii) For preparation of the optimal inoculum, 0.9 ml of the overnight *Candida* suspension (without agitation) was suspended in 360 ml of sterile water and subsequently mixed with 300 g of food powder. The median concentration of the *Candida* suspension was 6.0×10^6 CFU/ml (mean, 5.3×10^6; range, 2.17×10^6 to 8.0×10^6 CFU/ml). The mixture was spread on petri dishes as above and left to dry at 37°C for 48 h.

(iv) Food for control animals consisted of a mixture of 300 g of powdered chow and 360 ml of sterile water, which had been spread on sterile petri dishes and dried at 37°C for 48 h.

Chow containing *C. albicans* was stored in sterile plastic containers at room temperatures. Viability of *Candida* organisms in the chow was checked on the first and last days of feeding for every lot used. No significant differences were found in the concentration of *C. albicans* per gram of chow.

Food cultures. A 1-mg portion of dried food was homogenized in 9 ml of sterile isotonic saline (1:10 dilution) and vortexed for 2 min. A 100-μl amount of this suspension was inoculated onto SDA with cyclohexamide and chloramphenicol (BBL) to determine the number of viable *C. albicans* colonies in the food. Only concentrations of $\leq 10^9$ CFU/g of food were determined. Median yeast concentrations in the food were $>10^9$ CFU/g of food for the high inoculum, 2.5×10^4 CFU/g (mean, 2.1×10^4; range, 7.0×10^3 to 3.2×10^4 CFU/g) for the low inoculum, and 1.5×10^7 CFU/g (mean, 4.0×10^7; range, 7.5×10^6 to 1.0×10^8 CFU/g) for the optimal inoculum.

Feeding of mice. The 20 mice in each *Candida* group were fed 4- to 5-day-old chow containing *C. albicans* for 2 weeks. The mice in the control group were fed control chow during that period.

Stool cultures. Stools from each group of mice were cultured before and 14 days after initiation of the experimental diets. Quantitative cultures were also done at 48 h, 1 week, 1 month, and 3 months after completion of the diet on stools from Crl:CD1(ICR)BR mice. Cultures were done on stools from C3H/HeJ mice at the same intervals and also at 5 months postdiet. Stool specimens from each group of five mice (one stool pellet from each mouse) were homogenized and inoculated directly onto SDA with gentamicin (BBL) and SDA with cyclohexamide and chloramphenicol. Quantitative fungal stool cultures were prepared by homogenizing at 1:10 dilution of weighed stools in sterile isotonic saline and vortexing for 2 min. Tenfold serial dilutions were made, and 100 μl of each dilution was inoculated into each of the previously mentioned media. In addition, stools from mice fed the optimal inoculum were also cultured onto tryptic soy agar containing 5% sheep blood (Regional Media Laboratories), Tegretol 7 agar (Difco Laboratories), and Columbia CNA agar (Regional Media Laboratories), and quantitative stool cultures were prepared as described to characterize the normal gastrointestinal flora. Organisms were identified by the methods of Edwards and Ewing (6) and Cowan and Steel (4).

Evaluation of *C. albicans* dissemination. Fourteen days after initiation of the special diet, six randomly selected mice from each group were sacrificed by cervical dislocation and were dissected. The lungs, heart, liver, kidneys, and spleen were separately weighed and homogenized in 10 ml of saline, using a Stomacher Lab Blender 80 (Tekmar Co., Cincinnati, Ohio). A 100-μl amount of the suspension was then spread on plates containing tryptic soy agar with 5% sheep blood and SDA with cyclohexamide and chloramphenicol. Plates were incubated at 37°C for 48 h. Histopathologic examination was performed on lungs, liver, spleen, kidneys, adrenal glands, stomach, intestines, and mesenteric lymph nodes, with special emphasis on the detection of invasion of *C. albicans*.

RESULTS

Base-line stool cultures from control mice and mice to be fed with the optimal *Candida* inoculum yielded the same flora, including streptococci (mean concentration, 10^8 CFU/g of stool), *Escherichia coli* (10^5 CFU/g), *Bacillus* spp. (10^5 CFU/g), *Staphylococcus aureus* (10^4 CFU/g), *Acinetobacter calcoanitratus* serovar lwoffii (10^4 CFU/g), and unidentified molds (10^3 CFU/g). No base-line cultures yielded *C. albicans*. Stool cultures done 14 days after initiation of the experiments documented gastrointestinal *C. albicans* colonization in all *Candida*-fed mice. Control mice were not colonized with *C. albicans*. In the *Candida*-fed group, the median concentration of *C. albicans* in the stools was 10^8 CFU/g (mean, 2.0×10^8; range, 6.5×10^7 to 5.5×10^8 CFU/g) for the high inoculum, 1.8×10^3 CFU/g (mean, 5.9×10^3; range, 3.0×10^2 to 2.0×10^4 CFU/g) for the low inoculum, and 1.8×10^6 CFU/g (mean, 2.6×10^6; range, 9.2×10^5 to 5.9×10^6 CFU/g) for the optimal inoculum (Table 1). In mice fed with the optimal inoculum, the bacterial flora remained similar to the base-line flora in type and prevalence.

C. albicans was found in organ tissue cultures from mice in the high-inoculum group that were sacrificed on day 14 of the diet. The medians and ranges (in parentheses) of *C. albicans* concentration in organs (CFU per gram of tissue) were as follows: lungs, 400 (0 to 11,400); spleen, 160 (0 to 100,000); heart, 100 (0 to 2, 350); kidneys, 50 (0 to 14,000); and liver, 7 (0 to 1,100). Of six animals fed with the high inoculum, two failed to yield *C. albicans* on cultures of organs. No *C. albicans* was detected from the organ cultures of mice receiving low or optimum inocula. Organs from all remaining groups failed to grow the organism.

Histopathologic examination detected no evidence of infection with *C. albicans* even in the high-inoculum group. *Candida* organisms were only found in the intestinal lumen contents of mice fed with chow containing *C. albicans*, but there was no evidence of tissue invasion.

Stool cultures done 48 h after the end of the special diet showed a significant reduction (10- to 100-fold) in the concentration of *C. albicans*. Stool cultures done 1 week after stopping the diet yielded *C. albicans* in median concentra-

TABLE 1. Concentration of *C. albicans* determined by stool cultures[a]

Time period	*C. albicans* concn (CFU/g of stool)											
	Crl:CD1(ICR)BR, high inoculum			Crl:CD1(ICR)BR, low inoculum			Crl:CD1(ICR)BR, optimal inoculum			C3H/HeJ, optimal inoculum		
	Mean	Median	Range	Mean	Median	Range	Mean	Median	Range	Mean	Median	Range
Day 14 of diet	2.0×10^8	1.0×10^8	6.5×10^7–5.5×10^8	5.9×10^3	1.8×10^3	3.0×10^2–2.0×10^4	2.6×10^6	1.8×10^6	9.2×10^5–5.9×10^6	2.4×10^6	1.4×10^6	6.5×10^5–6.0×10^6
48 h after diet	1.3×10^6	1.4×10^6	4.5×10^5–2.0×10^6	0	0	0	2.7×10^4	1.0×10^4	7.8×10^3–8.0×10^4	2.1×10^5	1.6×10^5	8.3×10^4–4.5×10^5
1 wk after diet	2.2×10^4	2.3×10^4	1.0×10^4–3.4×10^4	0	0	0	2.8×10^3	2.7×10^3	8.0×10^2–5.1×10^3	3.1×10^4	2.7×10^4	7.5×10^3–6.2×10^4
1 mo after diet	4.3×10^3	2.6×10^3	1.0×10^3–1.1×10^4	0	0	0	2.9×10^3	2.5×10^3	5.0×10^2–6.1×10^3	1.7×10^4	1.4×10^4	5.2×10^3–4.1×10^4
3 mo after diet	2.2×10^3	1.6×10^3	9.0×10^2–4.8×10^3	0	0	0	2.9×10^3	2.3×10^3	1.0×10^3–6.0×10^3	2.3×10^4	2.3×10^4	6.5×10^3–4.1×10^4

[a] Base-line values were 0 in all cases. Values of 0 indicate that cultures did not reveal *Candida* spp. in the stools. Cultures from control mice [Crl:CD1(ICR)BR and C3H/HeJ] resulted in values of 0 at all phases of the diet.

tions of 2.3×10^4 CFU/g (mean, 2.2×10^4; range, 1.0×10^4 to 3.4×10^4 CFU/g) for the high-inoculum group and 2.7×10^3 CFU/g (mean, 2.8×10^3; range, 8.0×10^2 to 5.1×10^3 CFU/g) for the optimal-inoculum group. No *C. albicans* was recovered from stools of control mice or mice fed with the low inoculum. Stool cultures done 1 month after the end of the special diet showed that *C. albicans* persisted in the stools of the high- and optimal-inoculum groups at median concentrations of 2.6×10^3 and 2.5×10^3 CFU/g of stool, respectively. Stool cultures done 3 months after the end of the special diet demonstrated the presence of *C. albicans* at median concentrations of 1.6×10^3 CFU/g (mean, 2.2×10^3; range, 9.0×10^2 to 4.8×10^3 CFU/g) for the mice fed the high inoculum and 2.3×10^3 CFU/g (mean, 2.9×10^3; range, 1.0×10^3 to 6.0×10^3 CFU/g) for the mice fed the optimal inoculum (Table 1). No *C. albicans* was ever cultured from the stools of mice in the control group, and bacterial flora remained the same in the group of mice fed with the optimal inoculum. Also, no mice died during the 3-month follow-up period after the end of the special diet. Repeat experiments with the optimal-inoculum diet in C3H/HeJ mice produced results similar to those obtained with Crl:CD1(ICR)BR mice. The concentration of *C. albicans* in stools at 1 week, 1 month, and 3 months persisted at the level of 10^4 CFU/g (Table 1). Follow-up evaluations of C3H/HeJ mice after 5 months showed that the same level of gastrointestinal colonization was maintained.

DISCUSSION

In this study, we achieved *C. albicans* colonization of the gastrointestinal tracts of healthy adult mice without altering the host by feeding the mice chow containing *C. albicans* for 14 days. The fecal concentration of *C. albicans* was high at the end of the feeding period. The fungus persisted in the gastrointestinal tract in lower but stable concentrations for up to 5 months. Persistence of the fungus in the gastrointestinal tracts of two different breeds of mice confirms that this finding is not strain related.

Gastrointestinal colonization by *C. albicans* has been difficult to maintain in healthy adult mice without the use of immunosuppressive agents (8, 11, 23, 28). Prolonged colonization without the use of such agents has been achieved in infant and newborn mice (11, 12, 21–23). It is possible that the solid nature of the chow, which partially protects the fungus from gastric acidity, could have accounted for our ability to achieve this long-term *C. albicans* colonization. Most previously reported animal experiments used a suspension rather than a solid form of food containing *C. albicans* to feed the animals (7, 8, 14, 28). The role of gastric acidity in the prevention of *Candida* colonization and infection has been recognized previously. The reduction of gastric acidity by cimetidine is considered a likely explanation for the increased incidence of gastric and duodenal candidiasis in humans (26). Also, the inoculum size needed to induce disseminated candidiasis in animals was shown to be greater when the suspension containing *C. albicans* was delivered into the stomach rather than the small intestine. The activity of the gastric secretions might explain this difference (25).

One additional factor that may have contributed to colonization was the prolonged duration of feeding with the chow containing *C. albicans*. Previous studies used substantially shorter duration of feeding (≤3 days) (8, 14, 28). In the only study that achieved colonization after 3 days of feeding with a *Candida* suspension, sustained high-level colonization did not occur (8).

Persorption of *Candida* spp. through the intact intestinal mucosa and subsequent dissemination have been found to be related to the inoculum concentration (24). In our study, *Candida* dissemination did not occur when the *C. albicans* concentration in the consumed chow was $\leq 10^8$ CFU/g of food. There was a breakpoint in the concentration of the fungus in the feeding diet above which dissemination as well as colonization by the *Candida* spp. occurred. This result has been obtained in previous animal experiments and could be species and diet related (25). In our laboratory, using Crl:CD1(ICR)BR mice, this concentration has been determined to be $\geq 10^9$ CFU/g of food.

In conclusion, we have presented a simple and inexpensive model of producing *C. albicans* gastrointestinal colonization of mice. This model could be useful for understanding the pathophysiology of fungal gastrointestinal colonization in humans. It could also be used to mimic conditions in the immunocompromised host by treating the mice with antibiotics or immunosuppressive agents or both.

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