Gastric Colonization of *Candida albicans* Differs in Mice Fed Commercial and Purified Diets

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ABSTRACT It has been difficult to produce persistent colonization by *Candida albicans* in the gastrointestinal tract of adult mice without the use of antibiotics and immunosuppressants. We hypothesized that diet influences the colonization of *C. albicans* and tested the hypothesis. BALB/c mice fed either a commercial rodent diet or a nutritionally adequate mixture of purified ingredients were inoculated i.g. with *C. albicans* (5×10^7 cells). Gastrointestinal colonization was examined by fecal culture, tissue culture, and histology. Mice fed the purified diet had a high fecal recovery of *C. albicans* [5–6$\log_{10}$ colony forming units (cfu)/g feces] throughout the experimental period (6 wk), and the major site of colonization was the stomach. *C. albicans* was undetectable in the feces of mice fed the commercial diet 2 wk after inoculation. Immunosuppressants induced systemic dissemination of *C. albicans* in mice fed the purified diet. The number of lactobacilli and the concentration of organic acids in the stomach were significantly lower in mice fed the purified diet than in those fed the commercial diet. In vitro culture experiments revealed that acetic and lactic acids suppressed the growth of *C. albicans*. These results suggest that a reduction in lactobacilli in the stomach of mice fed the purified diet contributed to sustained gastric candidiasis. We therefore propose a novel model of sustained gastric candidiasis by a single i.g. inoculation of *C. albicans* in healthy adult mice fed a purified diet. J. Nutr. 135: 109–115, 2005.

KEY WORDS: • *Candidiasis* • *lactobacilli* • *purified diet* • *mice*

Candida albicans is a member of the indigenous microflora of the gastrointestinal (GI) tract and mucocutaneous membranes in healthy humans. However, it is also a potential pathogen and a frequent cause of complicating systemic infections and mortality in patients undergoing chemotherapy for cancer (1,2), immunosuppressive therapy (3), or prolonged antibiotic therapy (4). In addition, C. *albicans* was proposed to play a role in some cases of atopic diseases (5,6). Previous reports showed that antifungal drug therapy decreases both clinical scores and serum IgE levels in patients with atopic dermatitis (AD)[2] who displayed IgE-mediated hypersensitivity to C. *albicans* (7,8). C. *albicans* is rarely found in skin cultures (9,10), but is frequently found in fecal cultures of AD patients (11). Although these findings suggest a relation between GI candidiasis and allergic diseases such as AD, there are few experimental studies supporting this idea.

Most models for GI colonization of C. *albicans* were developed by oral inoculation of C. *albicans* in adult mice treated with antibiotics and immunosuppressive agents (12–17) or in infant mice (18,19). These treatments were necessary because competitive indigenous bacterial flora and the immune system prevent colonization by C. *albicans* (13,15,20). In light of the fact that C. *albicans* is an indigenous microorganism in the GI tract of healthy humans, however, such treatments should be avoided so that an animal model of GI candidiasis can be developed. This is especially true if immunosuppressive treatment seems unsuitable for establishing an animal model with which to study the relation between GI candidiasis and allergy.

We hypothesize that dietary components may be a determinant for colonization by C. *albicans* in the GI tract by influencing competitive indigenous bacterial flora and the growth of C. *albicans*. In the present study, therefore, we first investigated the GI colonization by C. *albicans* inoculated i.g. in mice fed a nutritionally adequate mixture of purified ingredients.

MATERIALS AND METHODS

Animals and diets. Male BALB/c mice (5 wk old), purchased from Charles River Japan, were housed in individual cages in a temperature-controlled (23 ± 2°C) room with a dark period from 1900 to 0500 h. They had free access to food and water. Mice were fed either a commercial rodent diet (MR stock; Nihon Nosan Kogyo)[3] or

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[2] Abbreviations used: AD, atopic dermatitis; BSA, bovine serum albumin; cfu, colony forming units; GI, gastrointestinal; YPD, yeast extract-peptone-dextrose.

[3] The commercial diet was composed of wheat bran, corn, oat bran, defatted soybean, wheat, fish meal, calcium carbonate, ground soybean, brewery yeast,

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TABLE 1

Composition of the purified diet

Ingredient	g/kg
α-Cornstarch[1]	529.5
Casein[2]	200.0
Sucrose[3]	100.0
Soybean oil[4]	70.0
Cellulose[5]	50.0
Mineral mix (AIN-93G-MX)[6]	35.0
Vitamin mix (AIN-93G-VX)[6]	10.0
L-Cystine[4]	3.0
Choline bitartrate[4]	2.5

[1] Purchased from Chuo-Shokuryou (Amylalpha CL).
[2] Purchased from New Zealand Dairy Board (ALACID).
[3] A gift from Nippon Beet Sugar.
[4] Purchased from Wako Pure Chemical Industries.
[5] Purchased from Advantec Toyo (Cellulose powder type D).
[6] Purchased from Nihon Nosan Kogyo. The mineral mixture and vitamin mixture are as reported in (21).

a purified diet (**Table 1**) prepared according to AIN-93G (21). This study was approved by the Hokkaido University Animal Use Committee, and mice were maintained in accordance with the guidelines for the care and use of laboratory animals of Hokkaido University.

Preparation of inoculum. *C. albicans* (ATCC 1542) was obtained from the Japan Collection of Microorganisms of The Institute of Physical and Chemical Research and maintained at 4°C on yeast extract-peptone-dextrose (YPD) agar. After culturing in YPD broth for 24 h at 37°C with orbital shaking at 100 rpm, organisms were harvested by centrifugation for 10 min at 3000 × *g*, washed once with sterile saline, counted by turbidimetry, and then adjusted with sterile saline to a concentration of 1×10^8 cells/mL.

Experimental design. In Expt. 1, mice weighing 20 ± 2 g were divided into 2 groups ($n = 9$) and fed either the commercial or purified diet for 2 wk. All mice were then deprived of food and water for 16 and 4 h, respectively, and then inoculated i.g. with 0.5 mL of saline containing 5×10^7 cells of *C. albicans*. Mice continued to consume their respective diets for 6 wk. Weekly fecal samples were collected and cultured for *C. albicans*, and blood samples were obtained from the tail vein for antibody measurements. On the last day of the experiment, mice were anesthetized by diethyl ether and killed by exsanguination from the carotid artery. After a thoracotomy and a laparotomy, the lungs, kidneys, stomach, small intestine, and colon were excised for enumeration of *C. albicans* as described below. For histologic examination, the stomach was excised, opened along the greater curvature, washed with ice-cold saline, and embedded in OCT compound (Sakura Finetechnical).

In Expt. 2, mice weighing 20 ± 2 g were divided into 2 groups ($n = 6$) and fed either the commercial or purified diet for 2 wk. All mice were inoculated with *C. albicans* as described above and continued to consume each diet. On d 42 and 45 after inoculation, mice were administered i.p. 150 mg cyclophosphamide monohydrate/kg body weight (Wako Pure Chemical Industries) and 65 mg prednisolone 21-hemisuccinate sodium salt/kg body weight (Sigma). On d 49, survivors were killed as described above, and the lungs, spleen, kidneys, liver, stomach, small intestine, and colon were then excised for enumeration of *C. albicans* as described below.

In Expt. 3, mice weighing 20 ± 2 g were divided into 2 groups ($n = 6$) and fed either the commercial or purified diet for 2 wk. All mice were then killed as described above, and the stomach was excised, weighed, and opened along the greater curvature. The gastric contents were subjected to bacteriological analysis and measurement of organic acids as described below. For histologic examination, the gastric wall was processed as described in Expt. 1.

calcium phosphate, salt, vitamins, and minerals. Nutritional values of the commercial diet are as follows (g/100 g): soluble nonnitrogen compounds, 56.6; protein, 18.7; water, 8.2; ash, 6.7, fiber, 5.7, and fat, 4.1.

Enumeration of C. albicans in feces and tissues. Fecal specimens were added to sterile PBS containing 100 kU/L penicillin and 100 mg/L streptomycin, homogenized using a polytron (Kinematica), and then cultured quantitatively by a standard pour plate technique. Briefly, fecal homogenates were diluted 10-fold with sterile PBS, and then 50 μL of each dilution was inoculated onto Candida GE Agar (Nissui Pharmaceutical). After 24 h of incubation at 37°C, the number of colonies was counted.

The lungs, kidneys, spleen, and liver were washed and homogenized in 2 mL of ice-cold sterile saline. The stomach was opened along the greater curvature, and the gross contents were removed gently with a spatula. The small intestine and colon were opened by a longitudinal incision, washed with ice-cold sterile saline to remove the gross contents, and cut into sections. Tissue samples from the stomach, small intestine, and colon were washed 3 times by vigorous agitation in 5 mL of ice-cold sterile saline in a plastic centrifuge tube and then homogenized in 2 mL of ice-cold sterile saline; 500 μL of each tissue homogenate was subjected to enumeration of *C. albicans* as described above.

Histology. Cryostat sections (5 μm) of the stomach were prepared and stained with hematoxylin and periodic acid-Schiff reaction for detection of *C. albicans* or with Gram stain for detection of tissue-associated bacteria.

Preparation of the cell wall fraction of C. albicans. The cell wall fraction of *C. albicans* was prepared for measurement of the *C. albicans*-specific antibody according to Mizutani et al. (22) with some modifications. Briefly, *C. albicans* harvested by centrifugation as described above was washed and incubated at a concentration of 5×10^9 cells/mL in sterile 50 mmol/L potassium phosphate buffer (pH 7.5) containing 1 mol/L NaCl, 0.3 g/L Zymolyase-20T (Seikagaku) and 1 g/L *Trichoderma* lysing enzymes (Sigma) at 37°C for 24 h with orbital shaking at 100 rpm Thereafter, the cell suspension was centrifuged at 3000 × *g* for 10 min, and the supernatant was collected as the cell wall fraction.

Assay for C. albicans–specific antibody. Serum samples were subjected to ELISA to measure serum levels of IgG, IgG1, and IgG2a specific to *C. albicans*. Microtiter plates (96-well; Corning) were coated with a portion of the cell wall fraction in 50 mmol/L carbonate buffer (pH 9.6) overnight at 4°C. After washing 2 times with PBS containing 0.02% Tween-20 (PBS-T), the plates were then blocked with 1% bovine serum albumin (BSA) in PBS-T for 2 h at 37°C. After washing 2 times with PBS-T, 1000-fold dilutions of sample sera or control sera from mice without inoculation were made with PBS containing 0.2% BSA and 0.02% Tween-20 (PBS-BT) and added to the wells, and the plates were then incubated for 1 h at 37°C. After washing 5 times with PBS-T, horseradish peroxidase-conjugated goat anti-mouse IgG (Zymed), rat anti-mouse IgG1 (Zymed) or rat anti-mouse IgG2a (Zymed) in PBS-BT was added and incubated at 37°C for 2 h. After washing 5 times with PBS-T, the plates were developed at room temperature after the addition of *o*-phenylenediamine (0.4 g/L) and hydrogen peroxide (0.016%) in 24 mmol/L citrate-50 mmol/L phosphate buffer (pH 5.0). Finally, 1 mol/L H_2SO_4 was added, and the absorbance was measured at 490 nm with a microplate reader (Model 550; Bio-Rad).

Bacteriological analysis of gastric contents. Bacteriological analysis of the gastric contents of mice was carried out according to the method of Mitsuoka et al. (23). Briefly, the fresh samples were diluted 10-fold with anaerobic phosphate buffer, and then 50 μL of each dilution was inoculated onto glucose-blood-liver agar for anaerobic bacteria and lactobacillus selection agar for lactobacilli. Anaerobic incubation was carried out at 37°C for 48 h by the steel-wool method, and aerobic incubation was carried out at 37°C for 48 h. The number of colonies was counted after the incubation.

Measurement of concentrations of organic acids in gastric contents. The concentrations of organic acids in the gastric contents of mice were determined using HPLC (Shimadzu) by the internal standard method. Gastric contents rinsed from the tissues were homogenized in 3 mL of ice-cold saline using a polytron, and 1 mL of the homogenate was added to 200 μL of 50 mmol/L sodium hydroxide aqueous solution containing 25 mmol/L crotonic acid (Wako Pure Chemical Industries) as an internal standard. After centrifugation at 13,000 × *g* for 10 min, the supernatant was extracted with chloro-

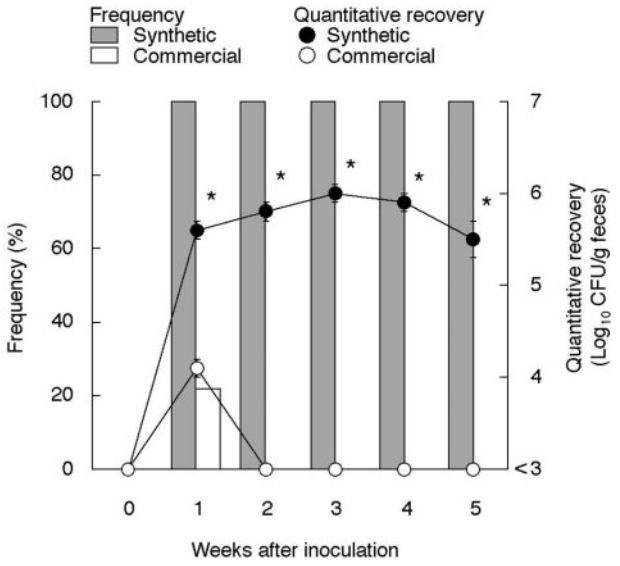

FIGURE 1 Changes in the frequency and recovery of organisms from the feces of mice fed the purified and commercial diets after an i.g. inoculation with *C. albicans*. Values are means ± SEM, $n = 9$. *Different from mice fed the commercial diet, $P < 0.001$.

form. The aqueous phase was passed through a 0.45-μm filter. The concentrations of individual organic acids (acetic, propionic, butyric, lactic, and succinic) in these samples were measured by ion-exclusion chromatography using a HPLC system equipped with a solvent delivery system (SLC-10 AVP; Shimadzu), a double ion-exchange column (Shim-pack SCR-102H, 8 × 300 mm; Shimadzu) and an electroconductivity detector (CDD-6A; Shimadzu) (24).

In vitro culture of C. albicans in the media supplemented with organic acids. *C. albicans* (initial concentration, 1×10^4 cells/mL) was cultured in Sabouraud Dextrose broth containing different concentrations of acetic acid and lactic acid at 37°C with orbital shaking at 100 rpm. The pH of the culture media was not adjusted. Amphotericin B (0.3 mg/L, Sigma) was used as a positive control for inhibition of *C. albicans* growth. After incubation for 0, 6, 12, 24, 36, and 48 h, the number of *C. albicans* colony forming units (cfu) was quantitated as described above.

Statistics. Results are presented as means ± SEM. To compare means of the 2 diet groups, data were analyzed by one-way ANOVA. To determine the effects of organic acids on the growth of *C. albicans*, data were analyzed by Tukey-Kramer's test after one-way ANOVA. StatView for Macintosh (version 5.0; SAS institute) was used for the analysis. Differences were considered significant at $P < 0.05$.

RESULTS

Gastrointestinal colonization of C. albicans. None of the mice fed the commercial diet had detectable *C. albicans* in feces 2 wk after an i.g. inoculation (**Fig. 1**). In contrast, all mice fed the purified diet had positive fecal cultures throughout the experimental period after inoculation, and the quantitative recovery of *C. albicans* from feces remained high (5–6 $\log_{10}$ cfu/g feces, **Fig. 1**).

At the time of killing, none of the mice fed the commercial diet had detectable organisms in their tissue cultures. In mice fed the purified diet, however, organisms were recovered from the stomach (5.5 $\log_{10}$ cfu/g tissue) but not from the small intestine or colon. In addition, none of the mice had detectable organisms in lung or kidney, suggesting no systemic dissemination of *C. albicans*.

Mice fed the purified diet showed histologic evidence of mucosal invasion by *C. albicans* on the forestomach adjacent to cardial-atrium line and the thick epithelium of forestomach (**Fig. 2***A*). Numerous yeast cells and hyphae in the gastric mucosa were observed at the site of *C. albicans* colonization (Fig. 2*B* and C). In contrast, mice fed the commercial diet showed no histologic evidence of the presence of *C. albicans* in the stomach (data not shown).

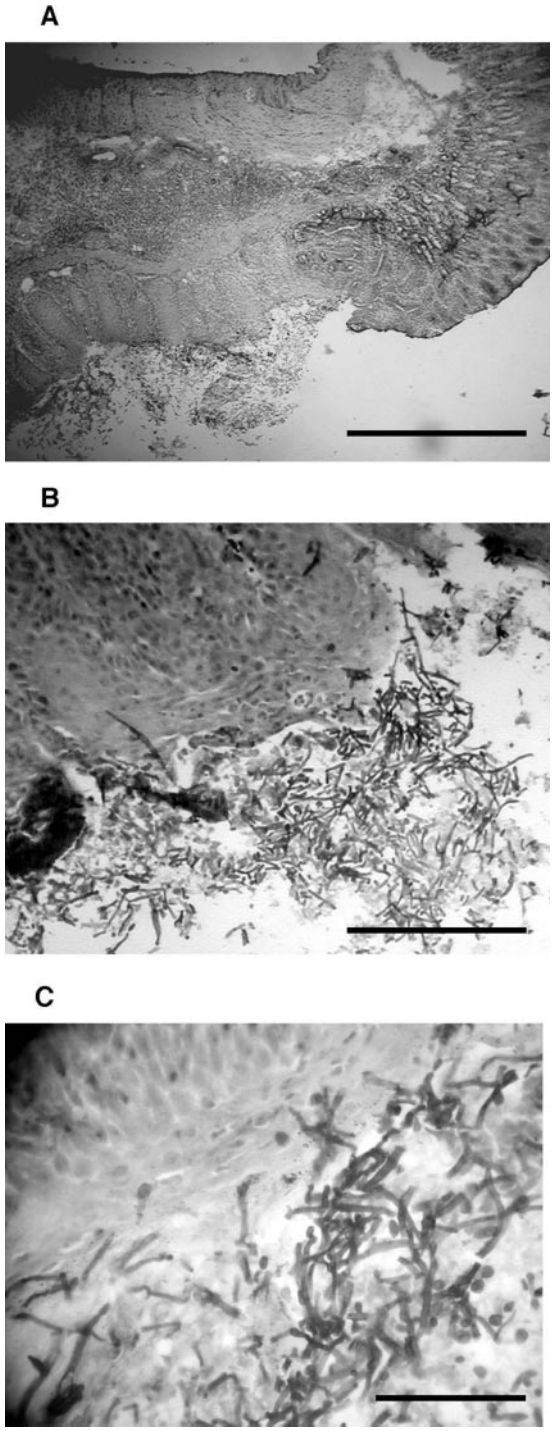

FIGURE 2 Histological sections of the gastric wall of a mouse fed the purified diet 6 wk after an i.g. inoculation of *C. albicans*. (*A*) The forestomach surface adjacent to cardial-atrium line was infected with *C. albicans*, and the thick epithelium of forestomach. Bar = 1 μm. Magnifications of *A* are shown in (*B*): bar = 100 μm and (*C*): bar = 20 μm. Yeast cells and hyphae were present in the surface area of the gastric wall. Periodic acid-Schiff and hematoxylin stain were used.

There was no detectable C. *albicans*-specific IgG in mice before inoculation (**Fig. 3**A). C. *albicans*–specific IgG levels were not elevated in mice fed the commercial diet throughout the experimental period. In contrast, C. *albicans*--specific IgG levels in mice fed the purified diet started to increase 4 wk after inoculation and continued to rise until they reached a plateau at 6 wk. At 6 wk after inoculation, C. *albicans*–specific IgG1 levels in mice fed the purified diet were higher than those in mice fed the commercial diet (Fig. 3B, $P < 0.05$). In contrast, C. *albicans*--specific IgG2a levels did not differ between the 2 groups (Fig. 3B).

Immunosuppression and systemic dissemination. Mice that had been fed the purified diet and administered the immunosuppressive agents 6 wk after inoculation with C. *albicans* had positive cultures for C. *albicans* in the GI tract and visceral tissues (**Table 2**). In contrast, in mice fed the commercial diet, organisms were detected in the stomach, but the level of colonization was much lower than that in mice fed the purified diet. Except for some organisms detected in the kidneys of 1 mouse, none were recovered from the visceral tissues of mice fed the commercial diet. Another experiment using mice administered immunosuppressive agents 2 wk after inoculation showed similar results (data not shown).

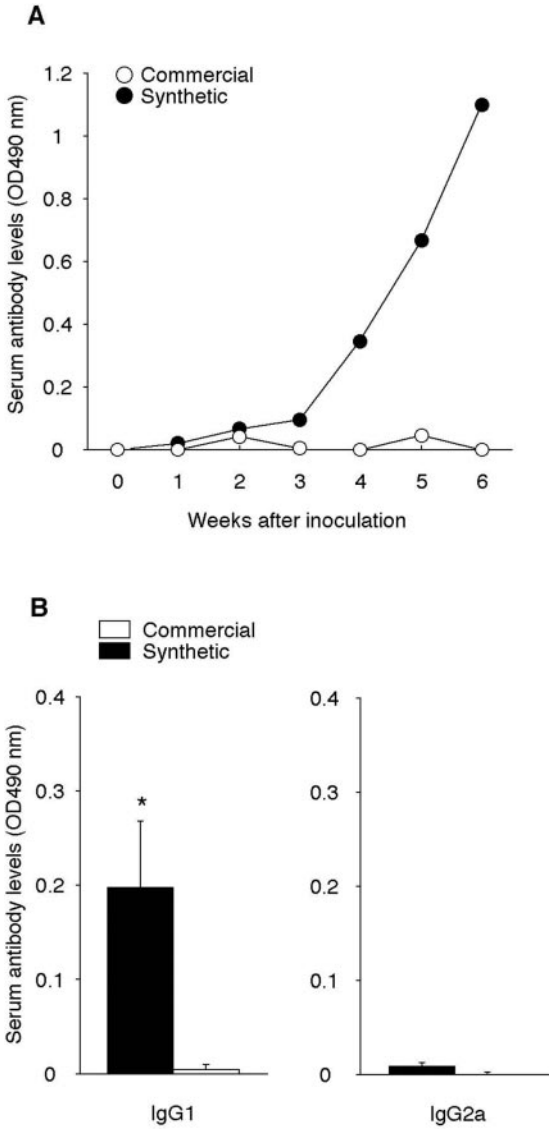

FIGURE 3 Serum levels of *C. albicans*–specific antibodies in mice fed the purified and commercial diets after an i.g. inoculation of *C. albicans*. (*A*) Changes in serum levels of *C. albicans*-specific IgG as measured in pooled serum, $n = 9$. (*B*) Serum levels of *C. albicans*-specific IgG1 and IgG2a on the last day of the experiment. Values are means ± SEM, $n = 6$. *Different from mice fed the commercial diet, $P < 0.05$.

TABLE 2

Quantitative cultures from tissues of mice fed the purified and commercial diets and administered cyclophosphamide and prednisolone 6 wk after inoculation with C. albicans[1]

	Purified diet		Commercial diet	
Tissue	Log_{10} CFU/g	Frequency	Log_{10} CFU/g	Frequency
Stomach	6.6 ± 0.1	6/6	3.7 ± 0.4	4/6
Small intestine	3.0 ± 0.2	5/6	ND[2]	0/6
Colon	4.0 ± 0.2	3/6	ND	0/6
Lungs	2.9 ± 0.1	6/6	ND	0/6
Spleen	3.3 ± 0.3	3/6	ND	0/6
Kidneys	2.2 ± 0.4	2/6	2.9	1/6
Liver	2.4 ± 0.1	6/6	ND	0/6

[1] Results are presented as quantitative recovery (means ± SEM of *C. albicans*-positive mice) and frequency (number of mice with positive culture/number of mice tested).
[2] ND, not detectable.

Bacterial populations and organic acids in stomach. Mice fed the purified diet had fewer anaerobic bacteria ($P < 0.01$) and lactobacilli ($P < 0.001$) in the stomach than mice fed the commercial diet (**Table 3**). Histologic examination revealed no bacterial layers on the epithelium of the stomach in mice fed the purified diet (**Fig. 4**A). In marked contrast,

TABLE 3

Populations of indigenous bacteria and concentrations of organic acids in the gastric contents of mice fed the purified and commercial diets[1]

	Purified diet	Commercial diet
	Log_{10} CFU/g contents	
Populations of indigenous bacteria[2]		
Anaerobes	7.2 ± 0.3	8.5 ± 0.2*
Lactobacilli	6.6 ± 0.3	8.5 ± 0.2**
	%	
Lactobacilli	38.3 ± 11.0	99.7 ± 0.2**
	μmol/g contents	
Organic acids		
Acetic acid	0.3 ± 0.2	5.3 ± 1.4*
Propionic acid	ND[3]	0.1 ± 0.1
n-Butyric acid	ND	0.8 ± 0.6
Lactic acid	7.2 ± 1.3	24.1 ± 5.5*
Succinic acid	0.2 ± 0.2	1.3 ± 0.3*

[1] Values are means ± SEM, $n = 6$. Asterisks indicate a difference from mice fed the purified diet * $P < 0.01$, ** $P < 0.001$.
[2] Aerobes were not detected.
[3] ND, not detectable.

dense layers of gram-positive bacteria were observed on the forestomach surface in mice fed the commercial diet (Fig. 4*B*). Most of the organisms in the bacterial layers in mice fed the commercial diet were gram-positive rods (Fig. 4C) and considered lactobacilli because lactobacilli were the most predominant bacterial species in the gastric contents (Table 3). Mice fed the purified diet had lower concentrations of organic acids in the gastric contents than mice fed the commercial diet (Table 3). In particular, the concentrations of acetic and lactic acids in mice fed the purified diet were lower than those in mice fed the commercial diet (Table 3, $P < 0.01$).

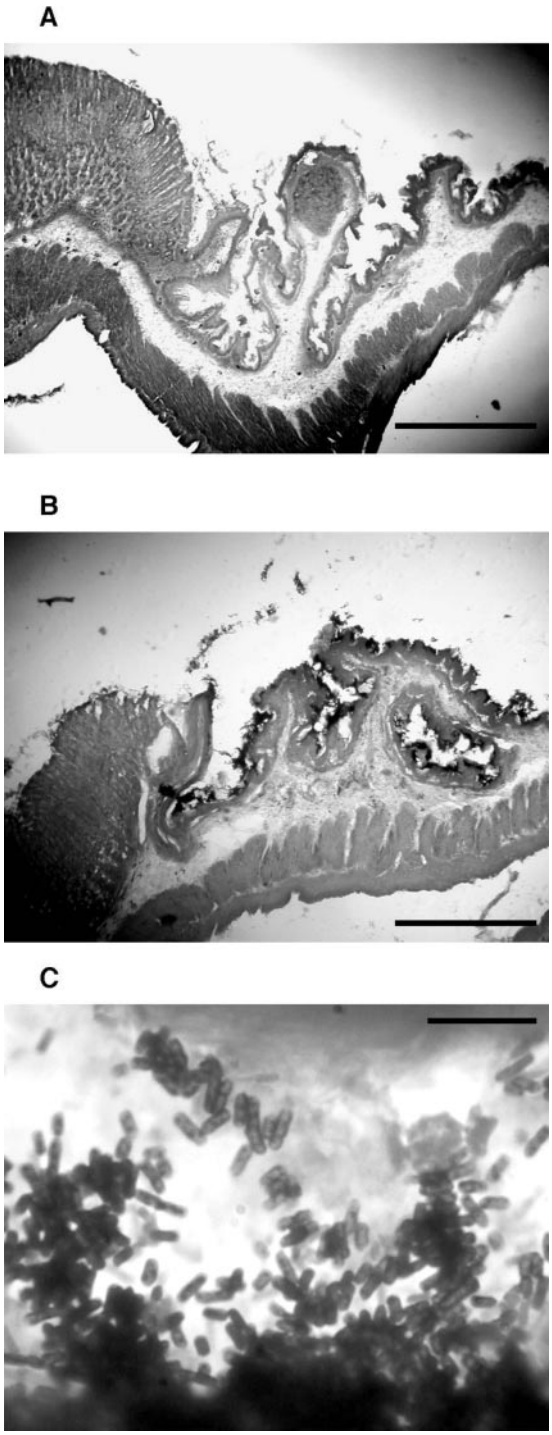

FIGURE 4 Histological sections of the gastric wall of mice fed the purified and commercial diets for 2 wk. (*A*) No layers of bacteria were observed on the stomach surface of mice fed the purified diet. Bar = 1 μm. (*B*) Dense layers of gram-positive bacteria were seen on the forestomach surface of mice fed the commercial diet; bar = 1 μm. (*C*) Higher magnification of (*B*): bar = 10 μm. Numerous gram-positive rods were present in the bacterial layer.

Inhibitory effect of organic acids on the growth of C. albicans. Acetic acid (**Fig. 5***A*) and lactic acid (Fig. 5*B*) dose-dependently suppressed the growth of C. *albicans* in vitro. In addition, although 20 mmol/L acetic acid (pH 4.5) and 100 mmol/L lactic acid (pH 3.3) had little effect on growth, the combination of 20 mmol/L acetic acid and 100 mmol/L lactic acid (pH 3.3) retarded the growth of C. *albicans* (Fig. 5C), suggesting a synergistic inhibitory effect of acetic and lactic acids.

DISCUSSION

Until now, sustained GI colonization of C. *albicans* was not successfully achieved by oral inoculation of C. *albicans* in healthy adult mice. This was attributed to the competitive indigenous microflora and the immune system, both of which could prevent colonization of C. *albicans* in the GI tract (13,15,20). Samonis et al. (25) reported that, in healthy adult mice fed a diet containing C. *albicans* ($>10^9$ cfu/g diet) for 2 wk, viable organisms were recovered from feces 48 h after the end of the treatment (5–6 $\log_{10}$ cfu/g feces), whereas the quantitative recovery of C. *albicans* from feces decreased after 4 wk (3–4 $\log_{10}$ cfu/g feces). In the present study, however, the quantitative recovery of C. *albicans* from the feces of healthy young-adult mice fed a nutritionally adequate mixture of purified ingredients, i.e., the AIN-93G diet, remained high (5–6 $\log_{10}$ cfu/g feces) throughout the experimental period after a single i.g. inoculation. In addition, in our preliminary experiments using healthy young-adult rats fed the purified diet, the quantitative recovery of C. *albicans* from feces remained high (4–5 $\log_{10}$ cfu/g feces) at least 11 wk after a single i.g. inoculation (unpublished data). In contrast, all mice fed the commercial diet had negative fecal cultures 2 wk after inoculation, in agreement with another published report (14). Thus, the unsuccessful colonization of C. *albicans* in the GI tract of healthy adult mice in previous studies was possibly due to feeding commercial rodent diets composed of natural materials. In other words, the present results suggest that feeding a purified diet would be an important determinant in producing sustained GI candidiasis in healthy adult mice.

In the present study, C. *albicans* colonized the forestomach surface adjacent to the cardial-atrium line in mice fed the purified diet. Previous reports also showed that the cardial-atrium line of the stomach was frequently colonized by C. *albicans* (14,26,27). Sustained gastric colonization of C. *albicans* in the present study was associated with a decreased number of lactobacilli in the stomach (Table 3, Fig. 4). Mice fed the commercial diet had a significantly greater number of lactobacilli and higher concentrations of organic acids in the stomach than mice fed the purified diet. Weak acids were used in the topical treatment of C. *albicans* infections (28), and the sensitivity of C. *albicans* to the acetic acid was reported (29). In the stomach of mice fed the commercial diet, organic acids produced by lactobacilli might suppress the growth of C. *albicans*. In fact, in vitro cultures of C. *albicans* showed that acetic and lactic acids inhibited the growth of C. *albicans*. The synergistic effect of the 2 acids may be due to an increase in undissociated acetic acid in the presence of lactic acid because the inhibitory effect of acetic acid on the growth of C. *albicans* *in vitro* was also increased by lowering the pH of the media with hydrochloric acid (data not shown). Because the concen-

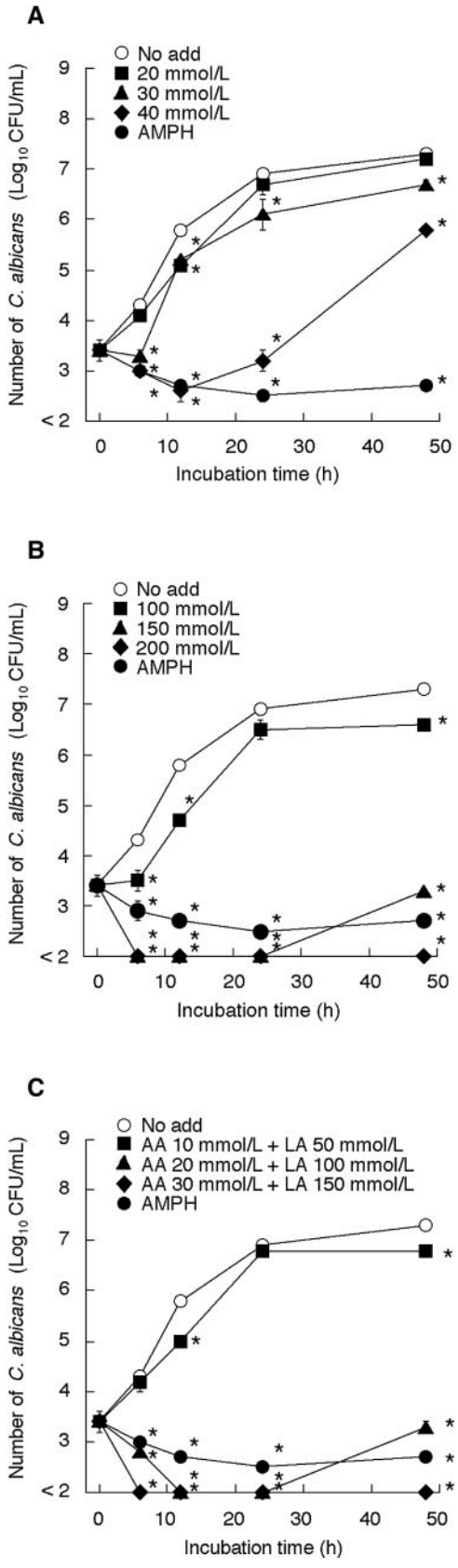

FIGURE 5 Growth curves of *C. albicans* cultured in Sabouraud Dextrose broth containing different concentrations of acetic acid (AA) (*A*), lactic acid (LA) (*B*), or both (*C*). Amphotericin B (0.3 mg/L) was used as a positive control for the inhibitor of yeast growth. Values are means ± SEM, $n = 3$. *Different from Sabouraud Dextrose broth alone, $P < 0.05$.

trations of organic acids showing the inhibitory effect in the present study appeared to be higher than those in the gastric contents in mice fed the commercial diet, the organic acids produced in the stomach of mice fed the commercial diet may be insufficient to suppress the growth of *C. albicans*. However, the concentrations of organic acids may be higher in the microenvironment near the epithelium than in the luminal contents of the stomach. Therefore, unsuccessful colonization of *C. albicans* in the stomach of mice fed the commercial diet could be due to the inhibitory effect of organic acids produced by lactobacilli in the forestomach.

The mechanism by which diets influence the number of lactobacilli in the stomach of mice remains unclear. *Lactobacillus* species generally demonstrate increased sensitivity at pH values <3, although differences exist between species and specific strains (30,31). However, the pH values of gastric contents in mice fed the purified diet and commercial diet were 4.0 and 4.3, respectively. Thus, the possibility that pH of the gastric contents is associated with the growth of the bacteria should be excluded. Brockett and Tannock (32) reported that the relative amounts of palmitic and oleic acids in the laboratory-prepared basic diet correlated with the number of tissue-associated lactobacilli in the stomach of mice. Additionally, they indicated that the commercially prepared pelleted food might contain substances that modified the toxic effect of the fatty acids against lactobacilli. Therefore, we speculate that the FFA composition of the diets would be a factor in influencing the number of lactobacilli in the stomach of mice. The biotherapeutic effect of lactobacilli on candidiasis in immunodeficient mice was reported (33). In addition, lactobacillus given to mice infected with *Helicobacter pylori* can eliminate the colonization of *H. pylori* in the stomach (34). Therefore, lactobacilli could be useful probiotics against *C. albicans* and *H. pylori*. Given that the FFA composition of the purified diet influences the growth of lactobacilli and that substances in the commercial diet modify the toxic effect of the fatty acids against lactobacilli, it would be possible to produce symbiotics from the substances and lactobacilli. Additionally, because dietary oligofructose and inulin were reported to protect mice from GI candidiasis (35), it is of interest to examine whether antagonism by lactobacilli is involved in the effect of these substances.

Mice fed the purified diet exhibited *C. albicans* colonization in the stomach but not the visceral tissues, suggesting that these mice are useful as an animal model mimicking healthy humans whose GI tract is colonized indigenously with *C. albicans*. *C. albicans* is an important opportunistic pathogen, causing systemic candidiasis in patients undergoing chemotherapy for cancer (1,2) and immunosuppressive therapy (3). Penetration of *Candida* species through the GI mucosa is thought to be the most frequent mechanism leading to systemic dissemination (36). To date, animals treated with antibiotics and immunosuppressive agents followed by oral inoculation were used to study systemic candidiasis arising from the GI tract (13,16). In the present study, treatment with immunosuppressive agents induced systemic dissemination of *C. albicans* in purified diet–fed mice in which *C. albicans* were colonized in the stomach. *Candida* infection involved all segments of the GI tract but was most common in the esophagus and stomach in immunosuppressed patients (2) and occasionally in otherwise apparently healthy persons (37,38). Therefore, immunosuppressed mice fed the purified diet used in the present study would be useful as an animal model with which to study the process of systemic candidiasis in an immunocompromised host.

In summary, we developed a novel model of sustained

gastric candidiasis by a single i.g. inoculation of C. *albicans* in healthy adult mice. The present model was successfully achieved by feeding a purified diet that reduced the number of lactobacilli in the stomach. We also showed systemic dissemination of C. *albicans* by immunosuppressive treatment. Our model would be useful for investigating not only antifungal compounds but also allergies against C. *albicans* and food ingredients.

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