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Pathogenicity of *Candida tropicalis* and *Candida albicans* After Gastrointestinal Inoculation in Mice

J. R. WINGARD,[1] J. D. DICK,[2] W. G. MERZ,[2]* G. R. SANDFORD,[1] R. SARAL,[1] AND W. H. BURNS[1]

The Johns Hopkins Oncology Center[1] and Department of Laboratory Medicine,[2] The Johns Hopkins Medical Institutions, Baltimore, Maryland 21205

The ability of clinical isolates of *Candida albicans* and *Candida tropicalis* to invade through normal and damaged gastrointestinal mucosa was determined. Adult mice were treated with either gentamicin or gentamicin and cytarabine. Suspensions of yeast cells (10^7) were administered through a catheter intraesophageally. Invasion was determined by culturing liver, kidney, and lung tissue from mice sacrificed after 48 h. *C. albicans* and *C. tropicalis* were incapable of invading through normal gastrointestinal mucosa in mice treated only with gentamicin. Two isolates of *C. tropicalis* penetrated the damaged gastrointestinal mucosa in 69% (49 of 71) of mice treated with gentamicin and cytarabine. In contrast, three isolates of *C. albicans* penetrated the damaged gastrointestinal mucosa in only 23% (14 of 62) of mice. These results suggest that *C. tropicalis* is more capable of invading through damaged gastrointestinal mucosa than *C. albicans*. The observations in this mouse model parallel those seen in patients on cytotoxic drugs. Therefore, this model offers a tool for investigation of the pathogenicity of these organisms in a model analogous to the compromised host.

We have recently reported that *Candida tropicalis* was the cause of the majority of fungal infections in granulocytopenic patients being treated for acute leukemia or undergoing bone marrow transplantation (18; G. R. Sandford, W. G. Merz, P. Charache, and R. Saral, Program Abstr. Intersci. Conf. Antimicrob. Agents Chemother. 18th, Atlanta, Ga., abstr. no. 98, 1978). This finding was strikingly different from earlier studies identifying *Candida albicans* as the major fungal pathogen in similar patient populations (3, 19). In our reports we noted that patients colonized with *C. tropicalis* were at significantly higher risk for infection than those colonized with *C. albicans*, suggesting a difference in pathogenicity. In contrast, previous studies examining differences in pathogenicity of *Candida* spp. in animal models generally found *C. albicans* to be more pathogenic than other species, including *C. tropicalis* (6, 8, 11). However, these animal studies used parenteral inoculation of the organisms and did not explore possible differences in the ability of the organisms to invade the gastrointestinal mucosa, a recognized portal of entry (5, 14-16). Such differences could be of major importance since cytoreductive agents and irradiation given to patients with acute leukemia and patients undergoing marrow transplantation are recognized to damage the gastrointestinal mucosa (2, 13) and could potentiate entry into the circulation. This communication describes a model for testing the comparative invasiveness of *Candida* spp. through the gastrointestinal tract of mice treated with antibiotics and cytoreductive therapy, a situation analogous to the compromised human host.

MATERIALS AND METHODS

Mice. Female DBA/2J mice weighing approximately 15 g were obtained from Jackson Laboratories, Bar Harbor, Maine, and used at 6 weeks of age.

Fungi. Three isolates of *C. albicans* and two isolates of *C. tropicalis* recovered from clinical specimens were maintained at 4°C on Sabouraud dextrose agar. Cultures of *C. albicans* were isolated from throat (JHH-U990), vascular-catheter tip (JHH-I995), and blood (JHH-M390) specimens. *C. tropicalis* isolates came from blood (JHH-A034) and sputum (JHH-N983). The two blood isolates were from patients who were proven to have had systemic infections, whereas the other isolates were from patients who were believed to be colonized but were without systemic fungal infections.

Yeast cells harvested from 24-h-old Sabouraud dextrose agar slants were inoculated into 300 ml of Sabouraud dextrose broth and incubated for 48 h with constant stirring at 35°C. The yeast cells were harvested by centrifugation, counted in a hemacytometer, and adjusted with physiological saline to a concentration of 2×10^8 per ml. Serial 10-fold dilutions were inoculated onto Sabouraud dextrose agar plates and incubated at 35°C for 48 h to determine the number of viable yeast cells.

Animal preparation. The preparative regimen is schematized in Fig. 1. Two days before administration of the fungus (day 1), polymixin B (50 mg/liter) was added to the drinking water. One day before yeast inoculation (day 2), gentamicin (20 mg/kg per day

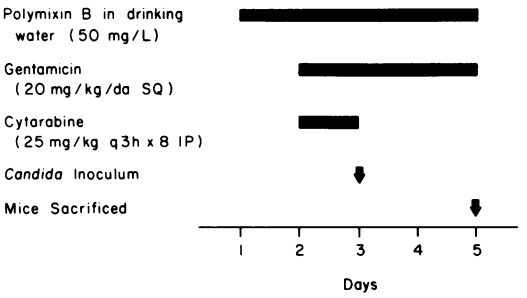

FIG. 1. *Preparative drug regimen. SQ, Subcutaneously; IP, intraperitoneally.*

subcutaneously) in three divided doses was begun and continued throughout the experiment. On day 2, cytarabine was also begun on a schedule described by Leach et al. (10) at a dose of 25 mg/kg per dose intraperitoneally at 3-h intervals for eight total doses.

Effects of preparative regimen. Control mice and cytarabine-treated mice were test bled from the retroorbital plexus at 48 h after completion of cytarabine (day 5). Leukocyte counts were determined manually with a hemocytometer after erythrocyte lysis. Blood smears were air dried and stained with Wright stain, and differential counts were performed. Sections of small intestine were fixed in 10% formaldehyde, imbedded in paraffin, cut by a microtome, and stained by hematoxylin and eosin in selected mice 24 h after completion of cytarabine (day 4).

Animal inoculation. An inoculum of 2×10^7 yeast cells in 0.1 ml was delivered per os intraesophageally by using a 1-ml tuberculin syringe and a Teflon catheter 3.2 cm in length (Travenol Laboratories, Inc., Deerfield, Ill.) 1 h after completion of the course of cytarabine (day 3).

In pilot experiments, animals were sacrificed at various intervals (3, 6, 12, 24, and 48 h) after intraesophageal yeast inoculation. Penetration of gut mucosa did not occur until 24 to 48 h after inoculation. Therefore, in subsequent experiments, mice were sacrificed and autopsied 48 h after yeast inoculation (day 5).

In further preliminary studies, the effect of intratracheal inoculation was evaluated to permit exclusion from analysis of those animals that received accidental pulmonary inoculation. Four groups of two mice each, control (normal) and cytarabine-treated mice, were anesthetized with pentabarbital. Their tracheas were exposed after skin incision and blunt dissection, and approximately 2×10^7 yeast cells of either *Candida* species in 0.1 ml of normal saline were injected into the trachea, using a tuberculin syringe with a 27-gauge needle. Mice inoculated in such a manner showed gross consolidation of the lungs at 48 h. Cultures of both control and cytarabine-treated mice lungs grew $>10^4$ colonies per mg of tissue of either *C. albicans* or *C. tropicalis*. In mice not receiving cytarabine, *C. albicans* was cultured only in the lungs; *C. tropicalis* was cultured not only in the lungs, but in low concentrations in the liver as well (<1 colony per mg of tissue). In cytarabine-treated mice, *C. albicans* was recovered in low concentrations (<1 colony per mg of tissue) in both liver and kidney, whereas *C. tropicalis* was additionally recovered from both liver and kidney in numbers comparable to the lungs ($>10^4$ colonies per mg of tissue). Based on these findings, mice inoculated per os found at sacrifice on day 5 to have $>10^4$ colonies per mg of lung tissue or those with yeasts in lung tissue alone were excluded from analysis, since intrapulmonary implantation could have occurred.

Approximately 10% of mice pretreated with antibiotics and cytarabine which were inoculated per os with either *Candida* species died within 24 h after inoculation. The lungs of these mice were consolidated at autopsy, and cultures grew $>10^4$ colonies per mg of tissue. These were also excluded from analysis since they were presumed to have aspirated yeast cells into the lungs.

The virulence of isolates after intravenous inoculation was determined by administration of yeast cells in 0.2 ml of physiological saline into the tail veins of mice, using a 1-ml tuberculin syringe with a 27-gauge needle. Groups of four mice each were given 10^6, 10^5, or 10^4 yeast cells, and mortality was observed for 30 days. The dose at which 50% of the mice died was calculated by the Reed-Muench method.

Tissue examination. Sections from liver, kidney, and lung tissue were weighed, suspended in 2.0 ml of Hanks balanced salt solution (Microbiological Associates, Walkersville, Md.), and homogenized by a motor-driven homogenizer (Bellco Glass, Inc., Vineland, N.J.). Undiluted and 1,000-fold diluted 0.1-ml samples of organ homogenates were streaked onto plates of Sabouraud dextrose agar plus gentamicin and incubated at 35°C for 48 h. The number of colonies was counted, and the number of viable organisms per milligram of organ tissue was determined.

RESULTS

Effects of preparative regimen. Figure 2 (A and B) shows a normal mouse small intestine (Fig. 2A) and the small intestine of a cytarabine-treated mouse (Fig. 2B) sacrificed 24 h after completion of cytarabine. The villi of the cytarabine-treated mouse were blunted, mitoses were reduced compared to the normal intestinal mucosa, karyorrhexis of crypt epithelial cells was present, and microabscesses with accumulations of granulocytes were scattered in the crypt areas. These changes were similar to those described by Leach et al. (10). The changes at 48 h were less striking. The average leukocyte count of four cytarabine-treated mice 48 h after completion of cytarabine was 1,137 (range, 800 to 1,700), and fewer than 10% were granulocytes. The average leukocyte count in four untreated control mice was 4,150 (range, 3,700 to 4,800) with 28% granulocytes.

Effects of intravenous yeast inoculation. The 50% lethal dose of *C. albicans* JHH-I995 was 6.7×10^5, that of *C. albicans* JHH-M390 was 1.7×10^4, that of *C. tropicalis* JHH-A034 was 4.2×10^4, and that of *C. tropicalis* JHH-N983 was 2.6×10^5.

Effects of gastrointestinal yeast inocula-

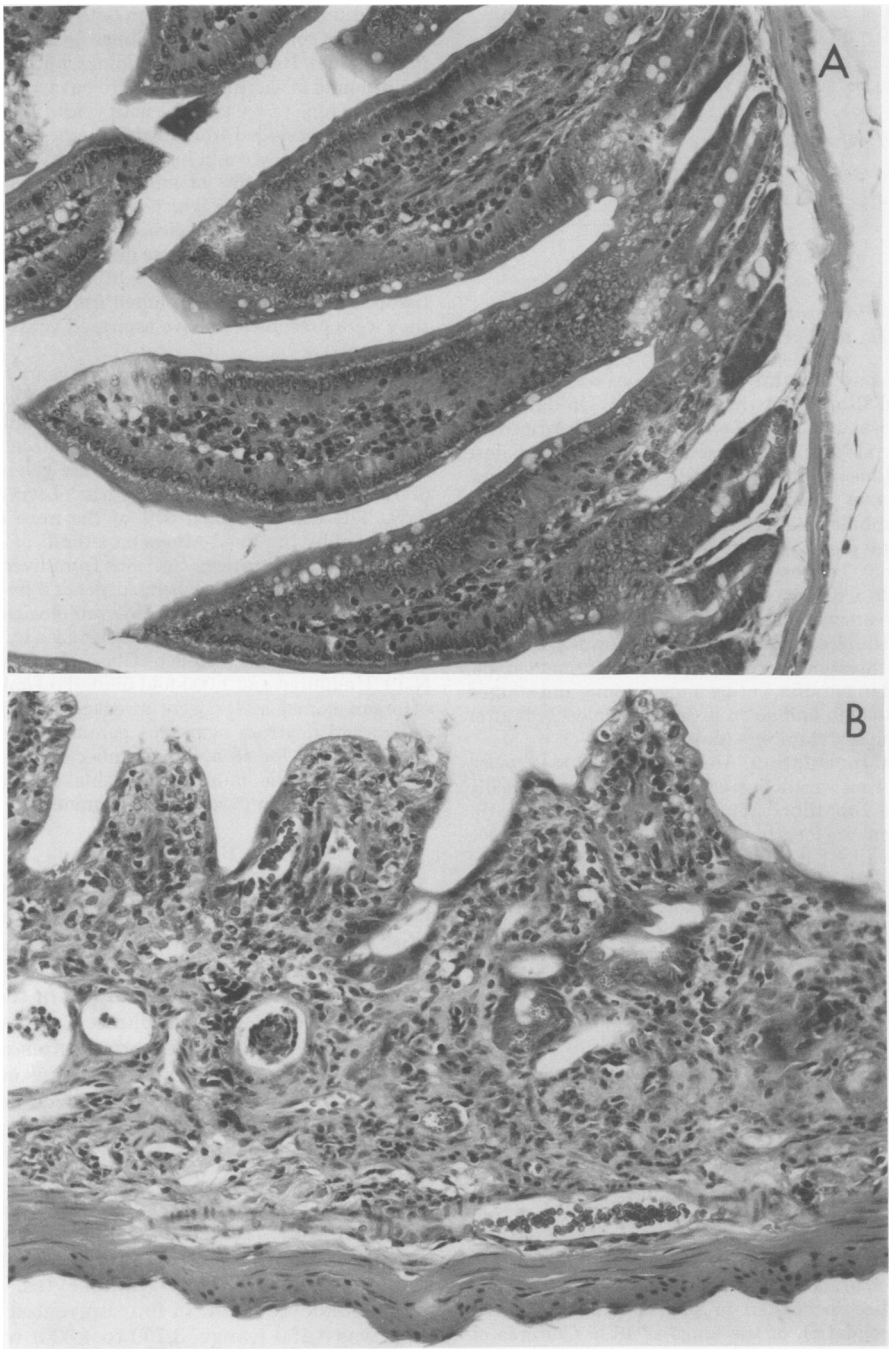

FIG. 2. *Intestinal tracts of mice stained with hematoxylin and eosin (×225). (A) Control animal; (B) 24 h after cytarabine treatment.*

tion. Control animals given various preparative regimens were cultured, as presented in Table 1. No yeasts were recovered from visceral organs of mice pretreated with antibiotics alone, cytarabine alone, or with antibiotics plus cytarabine. Visceral organ cultures of mice either given no pretreatment or treated with antibiotics and given gastrointestinal inoculation of either *C. albicans* or *C. tropicalis* were also negative.

Groups of mice treated with antibiotics plus

TABLE 1. *Recovery of C. albicans and C. tropicalis from visceral organs in control mice*

Regimen	Yeast inoculated	No. of viable yeasts inoculated	No. of mice from which viable yeasts were recovered/no. of mice studied
Antibiotics	None		0/8
Cytarabine	None		0/5
Antibiotics plus cytarabine	None		0/10
No pretreatment	*C. albicans* (JHH-I995)	1.3×10^7	0/5
No pretreatment	*C. tropicalis* (JHH-A034)	1.2×10^7	0/5
Antibiotics	*C. albicans* (JHH-I995)	1.3×10^7	0/10
Antibiotics	*C. tropicalis* (JHH-A034)	1.2×10^7	0/9

cytarabine were inoculated with two isolates of *C. tropicalis* and three isolates of *C. albicans*. In contrast to the consistently negative cultures of visceral organs in the earlier experiments, positive cultures were found (Table 2). Although positive cultures were noted in both *C. albicans*- and *C. tropicalis*-inoculated mice, only 23% (14 of 62) of mice inoculated with *C. albicans* had positive visceral organ cultures, whereas 69% (49 of 71) of mice inoculated with *C. tropicalis* had positive visceral organ cultures. This is a statistically significant difference ($P < 0.001$). Tenfold increases in the inoculam of *C. albicans* (JHH-U990) and *C. tropicalis* (JHH-A034) significantly increased the number of animals with positive visceral organ cultures ($P < 0.05$).

There were no substantial quantitative differences in recovery from liver (6.8 ± 2.3 colonies per mg of tissue), lung (10.2 ± 4.4 colonies per mg of tissue), or kidney (4.1 ± 1.6 colonies per mg of tissue) in mice from which *C. tropicalis* was recovered. Mice inoculated with *C. albicans* showed similar organ distribution when invaded but in smaller quantities in each visceral organ.

DISCUSSION

No visceral organ invasion by either *C. albicans* or *C. tropicalis* was noted in our normal mice. This was probably due in part to the mouse's intact gastrointestinal mucosa acting as an effective barrier in the prevention of yeast invasion. However, the inoculum size was probably also contributory since others have shown that *C. albicans* can invade through the normal intact gastrointestinal mucosa when given in substantially greater inocula (9, 16). Alteration of the microbiological flora by prior antibiotic treatment was insufficient to permit visceral invasion in our study conditions, and this concurs with earlier studies (4, 7, 17).

Visceral invasion by both *Candida* species occurred when mice were pretreated with a cytoreductive agent and antibiotics. We chose cytarabine in our model since this (cell cycle-specific) inhibitor of deoxyribonucleic acid synthesis is a major drug commonly used in the treatment of acute myelogenous leukemia, and because dose schedules for this drug had been previously tested in mice (10). Treatment with cytarabine resulted in granulocytopenia and gastrointestinal mucosal damage, as noted in earlier studies (10) and confirmed in this study. Oral polymixin B and parenteral gentamicin were also included in the drug regimen because leukemic patients usually receive oral nonabsorbable antibiotics during granulocytopenia and virtually all receive systemic antibiotics, usually including an aminoglycoside. This drug combination provided for major changes in the host mouse model. Cytarabine resulted in a disruption of the mechanical and physiological gastrointestinal barrier with the concurrent elimination of a major systemic host defense, granulocytes. Although antibiotics did not permit invasion, they may have permitted fungal overgrowth through the suppression and alteration of resident bacterial flora and prevented rapid, fulminant bacterial sepsis via a damaged gastrointestinal tract. These host alterations parallel the impaired host defenses in

TABLE 2. *Recovery of Candida organisms from visceral organs of mice treated with antibiotics and cytarabine*

Yeast inoculated	No. of viable yeast inoculated	No. of mice from which viable yeasts were recovered/no. of mice inoculated
C. albicans		
JHH-U990	3.1×10^6	0/15
	4.8×10^7	1/8
	1.2×10^8	6/11
JHH-I995	1.2×10^7	4/13
	7.1×10^7	2/6
JHH-M390	3.0×10^7	1/9
		14/62[a]
C. tropicalis		
JHH-A034	1.8×10^7	6/15
	1.3×10^7	12/16
	6.8×10^7	23/26
JHH-N983	8.0×10^6	8/14
		49/71[a]

[a] $P < 0.001$.

the human population at highest risk for disseminated candidiasis: patients with severe bone marrow failure or those undergoing cytoreductive treatment for hematological malignancies.

Other animal studies have shown gastrointestinal invasion by *C. albicans*. As in our investigation, some alteration generally was present before visceral invasion: prior irradiation, antibiotics, and corticosteroids (17), immaturity of host defenses (neonates) (12), or nutritional deficiency (1). Similarly, in human studies with compromised patients the gastrointestinal tract has been shown to be a major portal of entry for disseminated candidiasis (5, 14–16).

In using the model to study two common yeast isolates, a striking difference in the ability of *C. albicans* and *C. tropicalis* to penetrate the gastrointestinal mucosa and cause systemic infection became apparent. To our knowledge, no previous studies have examined comparative pathogenesis for different strains and species of *Candida* after gastrointestinal inoculation. The observation that *C. tropicalis* is more pathogenic than *C. albicans* is further supported in recent reports from this institution studying compromised humans (18; Sandford et al., 18th ICAAC, abstr. no 98). In these studies, the incidence of systemic *C. tropicalis* infection was consistently higher than *C. albicans* infection despite higher gastrointestinal colonization rates for *C. albicans*. Possible explanations for this divergence include strain or species differences in the capacity to traverse the damaged gastrointestinal tract or differences in the ability of yeasts to withstand remaining host defenses in the immunocompromised subject. Although the sample size is small, the *C. albicans* and *C. tropicalis* used in the development of the mouse model included both a blood isolate (from a patient with systemic infection) and a superficial (noninfecting) isolate for each species, with no apparent differences noted between isolates of the same species.

It is noteworthy that in prior studies of comparative virulence after intravenous inoculation, *C. albicans* has been found to have a pathogenicity equivalent to, or greater than, that of *C. tropicalis* (6, 8, 11). We also documented that the isolates of both *Candida* species in our study had comparable virulence after intravenous inoculation as measured by 50% lethal dose. We feel that the studies using intravenous inoculation may not be comparable to the typical human situation since the animal hosts were not compromised in a manner that paralleled the human experience. Furthermore, the findings of these studies with the systemic circulation as the portal of entry would not demonstrate differences in capacity to gain entrance to the circulation through a mucosal barrier. Thus, it appears that virulence characteristics determined by the two models are different. Presumably, lethality after intravenous inoculation into a normal host provides a measure of tissue destructiveness, whereas invasion after gastrointestinal inoculation of a compromised host provides a comparative measure of mucosal barrier penetration in addition to a measure of tissue destruction.

This model offers a rapid and reproducible system to test the virulence of *Candida* spp. which correlates well with human observations. With the observation of significant species differences in causing systemic infection, further investigation is required to elaborate the specific mechanisms and determinants involved in systemic fungal infection originating in the gastrointestinal tract of immunocompromised hosts receiving antibiotics and chemotherapy.

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