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Candida tropicalis Infection in Normal, Diabetic, and Neutropenic Mice

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Opportunistic infections caused by *Candida tropicalis* have been noted with increasing frequency in compromised patients. The pathogenicity of three isolates of *C. tropicalis* was studied in normal CD-1 mice, streptozotocin-induced diabetic mice, and cyclophosphamide-induced neutropenic mice. Lethal dose 50% endpoints and quantitative distribution of yeast cells in spleen, liver, and kidneys of mice infected intravenously were determined. The virulence of one yeast isolate was greater than that of the other two. The order of susceptibility to mortality and degree of organ colonization was neutropenic > diabetic > normal. Renal lesions resembling those associated with infection by *C. albicans* appeared by day 5 postinfection in diabetic and neutropenic mice. Greater numbers of *C. tropicalis* yeast cells were isolated from homogenates of the affected kidneys, suggesting that the kidney is a target organ for this fungus. This study demonstrates the increased susceptibility of compromised mice to *C. tropicalis* as compared with normal mice and verifies the ability of these yeasts to cause opportunistic disease.

The term candidiasis often is used to describe an infection caused by the yeastlike fungus *Candida albicans*. Species of *Candida* other than *C. albicans*, however, also have the potential to cause infection, particularly in patients who are immunologically or physiologically compromised (7, 12, 13, 20).

Candida tropicalis has emerged as a potentially dangerous opportunistic fungus. This may be due both to an increased awareness and specific identification of *C. tropicalis* as an etiologic agent of infection and to an increase in the number of compromised patients susceptible to opportunistic fungi. In one study, *C. tropicalis* was the most frequent opportunistic fungus isolated from specimens from patients in a critical care unit (12). *C. tropicalis* also has been reported to be a frequent opportunistic pathogen in a cancer hospital (7) and has been identified as the etiologic agent in a variety of infections including pyelonephritis (5, 14, 17), lower urinary tract infections (5), thrombophlebitis (4), arthritis (10, 16), bursitis (16), meningitis (3), multiple organ infection (1), pericarditis (6), and candidal vulvovaginitis (8).

The purpose of this study was to develop and characterize experimental models of *C. tropicalis* infection with the goal of learning more about the virulence and pathogenicity of this fungus in different physiologic and immunologic populations of mice. These experiments were designed to answer three questions: (i) are there differences among three strains of *C. tropicalis* in their lethality and virulence; (ii) are there differences in infection among normal, diabetic, and neutropenic mice; and (iii) is there a difference in colonization of *C. tropicalis* among major organs?

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MATERIALS AND METHODS

Fungi. Three human isolates of *C. tropicalis* (Merck Culture Collection strain numbers MY1124, MY1162, and MY1163) were used. The isolates were maintained on Sabouraud dextrose agar (Difco Laboratories, Detroit, Mich.) and subcultured routinely. Identifications of each isolate were verified by using the API 20C yeast identification system (Analytab Products, Inc., Plainview, N.Y.).

Animals. CD-1 female mice (Charles River Breeding Laboratories, Inc., Wilmington, Mass.) weighing 20 g were used in the study. Normal and diabetic mice were housed in standard boxes with corncob bedding and given food and water ad libitum. Neutropenic mice were housed in microisolator boxes containing sterilized corncob bedding and given sterilized food and water ad libitum. Mice were housed five per box.

Induction of diabetes. Mice were injected intravenously (i.v.) with 200 mg of streptozotocin (Calbiochem-Behring, La Jolla, Calif.) per kg. One week after injection of streptozotocin, the urine of each mouse was tested for the presence of glucose with Clinistix Reagent strips (Ames Div., Miles Laboratories, Inc., Elkhart, Ind.). Animals with urine glucose levels >500 mg/dl, i.e., with glucosuria, were used for the study. Preliminary experiments verified that blood glucose levels of the mice were >250 mg/dl as determined by a colorimetric assay (Sigma Chemical Co., St. Louis, Mo.); these glucose concentrations are indicative of severe diabetes.

Induction of neutropenia. Mice were injected intraperitoneally with cyclophosphamide (CY; Cytoxan; Bristol Laboratories, Syracuse, N.Y.) at a dose of 100 mg/kg. One injection per day for five consecutive days was administered. Leukocyte and differential counts from peripheral blood were monitored for up to 16 days to verify the induction of neutropenia.

Determination of lethal dose endpoints. Titrated doses (inoculum determined by hemacytometer counts) of each

TABLE 1. LD_{50}s of three isolates of *C. tropicalis* in normal, diabetic, and neutropenic CD-1 mice[a]

C. tropicalis isolate	LD_{50}		
	Normal mice	Diabetic mice	Neutropenic mice
MY1124	1.45×10^7	5.37×10^5	1.86×10^4
MY1162	2.02×10^6	2.24×10^4	1.26×10^4
MY1163	2.40×10^7	6.46×10^4	4.53×10^3

[a] The LD_{50} is the concentration of cells required to kill 50% of infected mice 14 days after i.v. infection.

strain of *C. tropicalis* were inoculated i.v. into the lateral tail veins of normal, diabetic, and neutropenic mice. The animals were monitored for 14 days postinfection (p.i.), and mortality was recorded. Animals that died were autopsied and cultures were obtained to confirm the presence of *C. tropicalis*. Lethal dose 50% endpoints (LD_{50}s) were calculated at 14 days by the method of Knudsen and Curtis (10). Experiments were done in triplicate.

Determination of CFU in organs. In a separate series of experiments designed to determine the extent of *Candida* invasion of organs, groups of normal, diabetic, and neutropenic mice were infected i.v. with 10^5 viable yeast cells of each isolate of *C. tropicalis*. On days 1, 2, 5, 7, and 9 p.i., two mice per experimental group were selected at random and sacrificed. The peritoneal and thoracic cavities were exposed and examined for evidence of infection, e.g., lesions or granulomas. Both kidneys, the liver, and the spleen were removed aseptically and transferred to sterile polyethylene bags (Whirl-Pak; Ace Scientific, Linden, N.J.) in which they were homogenized in 5 ml of sterile saline. Samples were removed from each homogenate, serially diluted 10-fold in sterile saline, and plated onto Sabouraud dextrose agar. After incubation at 25°C for 48 h, the colonies were enumerated, and CFU were calculated for each organ. All experiments were performed in duplicate, so that CFU reported are based on four mice per experimental group, a number quite sufficient for accurate statistical analysis as outlined below. All kidney data are from paired kidneys.

Statistical analysis. All data were analyzed by a multivariate analysis of variance (MANOVA). This multivariate analysis allowed for the correlation among CFU obtained from organs within the same animal. The primary response variable considered throughout was $\log_{10}$ of the *C. tropicalis* CFU. All statistical tests were performed at the 5% significance level.

TABLE 2. Results of the MANOVA for *C. tropicalis* CFU data[a]

Source of variation	*P* value[b]
Strain	0.0002
Treatment	<0.0001
Day	<0.0001
Strain by treatment	0.68
Strain by day	0.09
Treatment by day	<0.0001
Strain by treatment by day	0.57

[a] The response variable for this analysis is the three-dimensional vector consisting of $\log_{10}$ CFU in the kidneys, liver, and spleen, respectively.

[b] The *P* value is for testing the hypothesis that the given factor has no effect on mean response. The smaller the *P* value, the stronger the evidence that the factor does indeed have an effect. *P* values reported here are based on Wilks's lambda criterion, a standard test statistic in the MANOVA.

TABLE 3. *C. tropicalis* CFU data: response means by strain[a]

Treatment group	Mean response ($\log_{10}$ CFU ± SEM[b]) in:			
	Kidneys	Liver	Spleen	All three organs
MY1124	4.31 ± 0.25	2.52 ± 0.18	2.24 ± 0.16	3.02 ± 0.15
MY1162	4.97 ± 0.25	3.12 ± 0.19	2.51 ± 0.18	3.53 ± 0.17
MY1163	4.40 ± 0.22	2.41 ± 0.20	1.87 ± 0.16	2.90 ± 0.13

[a] The response variable is $\log_{10}$ CFU. Responses have been averaged over all 5 days of measurement and over all treatment groups.

[b] Standard errors of the relevant means. These standard errors do not reflect the correlation among means for the three organs.

RESULTS

Induction of neutropenia. Preliminary experiments verified that severe neutropenia was achieved by the injection of CY. Within 2 days of the initial CY injection, spleen weights and cellularity were markedly decreased. Peripheral leukocyte counts fell as low as 500 cells per mm^3 by day 4 of the study, and differential counts of blood smears revealed substantial reduction of polymorphonuclear cells. Leukocyte counts and differential profiles in the CY-treated mice returned to normal by day 10 of the study. Leukocyte counts and differential profiles were not affected by streptozotocin-induced diabetes.

Differences in virulence of *C. tropicalis* isolates. The LD_{50}s for the three strains of *C. tropicalis* in the normal, diabetic, and neutropenic mouse models are listed in Table 1. The LD_{50} data represented 14-day tests and suggested that strain MY1162 was the most lethal strain as measured by mortality. This was confirmed when the combined CFU data from kidneys, spleen, and liver were evaluated by a MANOVA (Tables 2 and 3). When the data for the three strains were analyzed across time, at day 1 p.i., all three strains had comparable levels of infection but, by day 5 p.i., strain MY1162 yielded CFU that were considerably higher (approximately 0.6 units on a $\log_{10}$ scale) than for the other two strains ($P < 0.05$) (Table 3). While differences in infection levels fluctuated over time, average CFU for MY1162 were consistently higher on and beyond day 5 p.i.

Average CFU for the other two strains were comparable to each other throughout the test period. Differences in strain virulence were consistent across organs and across treatment.

Differences among treatment groups (normal, diabetic, and neutropenic). Testing for differences among the three treatment groups was done by the MANOVA (Table 2). In this analysis, the focus was on testing for an overall treatment effect and for interactions of such an effect with the other factors, strain, and day. The MANOVA showed that a significant difference in the susceptibility of each group to

TABLE 4. *C. tropicalis* CFU data: response means by treatment group[a]

Treatment group	Mean response ($\log_{10}$ CFU ± SEM[b]) in:			
	Kidneys	Liver	Spleen	All three organs
Normal	3.15 ± 0.16	1.78 ± 0.18	2.06 ± 0.14	2.33 ± 0.11
Diabetic	5.03 ± 0.22	2.77 ± 0.13	1.78 ± 0.16	3.19 ± 0.11
Neutropenic	5.51 ± 0.22	3.50 ± 0.20	2.78 ± 0.18	3.93 ± 0.17

[a] The response variable is $\log_{10}$ CFU. Responses have been averaged over all 5 days of measurement and over all three strains.

[b] Standard errors of the relevant means. These standard errors do not reflect the correlation among means for the three organs.

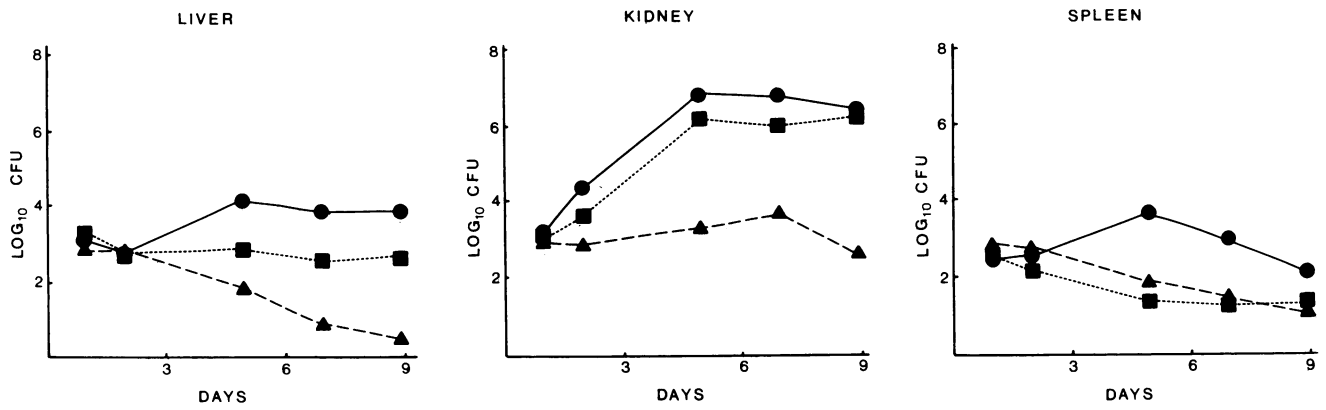

FIG. 1. CFU of *C. tropicalis* obtained from the liver, spleen, and kidneys of normal, diabetic, and neutropenic mice. The data are organized and illustrated for each experimental group. Symbols: ▲, normal; ■, diabetic; ●, neutropenic.

infection existed ($P < 0.0001$). The existing differences in susceptibility of the three treatment groups were consistent across the three strains of *C. tropicalis* used; that is, there was no significant strain-by-treatment interaction ($P = 0.68$).

Overall, the neutropenic mice had significantly higher mean CFU in the organs examined than either the normal or diabetic groups ($P < 0.01$) (Table 4). Except for day 1 p.i., the neutropenic mice had significantly higher CFU than either of the other two groups (Fig. 1). From day 5 p.i. on, the diabetic group had significantly higher infection levels than the normal group ($P < 0.01$) (Fig. 1). The pattern of differences in CFU per day among the treatment groups varied according to organ (Fig. 1). Patterns for the spleen and liver were similar to each other; data from the kidneys were greater in magnitude and different in kinetics when compared with the data obtained from the spleen and liver (Fig. 1). Specifically, in the kidney, the difference in mean $\log_{10}$ CFU between diabetic and normal mice exceeded that observed between the neutropenic and diabetic groups; this was not observed in the data from the spleen and liver. The order of group susceptibility to extent of organ colonization was neutropenic > diabetic > normal.

Differences among colonization of organs. Upon gross examination, kidneys heavily infected with *C. tropicalis* were characteristically edematous and hemorrhagic with numerous subcapsular petechial lesions representing foci of yeast cells and hyphae; the grossly affected kidneys occurred by 5 days p.i. in both diabetic and neutropenic mice. Only a few normal mice developed kidney infections severe enough to have petechial lesion manifestations. Testing for differences in colonization of the kidneys, spleen, and liver of mice from the three treatment groups was performed by MANOVA techniques. When the multivariate analysis provided evidence for a statistical effect, more detailed univariate analyses were done to clarify the observed effect further. Analysis of the data indicated that significant differences in the degree of organ colonization existed ($P < 0.0001$). From day 2 p.i. onward, infection in the kidney, as measured by CFU, was significantly higher than that of the liver and spleen, suggesting that the kidney is the target organ for *C. tropicalis* infection administered i.v. (Table 5). Colonization of the liver was significantly higher than that of the spleen on average, although the difference between these two organs was not as large as that observed between them and the kidneys. Differences in colonization among organs varied with treatment ($P < 0.0001$) and with time ($P < 0.0001$). Differences between kidney and liver and between kidney and spleen CFU were far more pronounced in the neutropenic and diabetic mice than in the normal mice (Fig. 2). This is partly attributable to the fact that colonization in the kidneys of the normal group never attained levels comparable to those in the diabetic and neutropenic mice (Fig. 2). The observed differences among organs were consistent across the three strains as evidenced by a nonsignificant organ-by-strain interaction ($P = 0.22$). The order of extent of colonization of organs for all three fungal strains and experimental groups was kidney > liver > spleen.

DISCUSSION

C. tropicalis is considered to be an opportunistic, yeastlike organism which is common both in the environment and as a human commensal organism. Under certain conditions, *C. tropicalis* has been shown to cause significant morbidity and mortality (1, 3–8, 11, 12, 14, 16, 17, 20) and has been described as a major pathogen in immunocompromised patients (20).

In the present study, we analyzed colonization of kidneys, liver, and spleen by three strains of *C. tropicalis* after i.v. inoculation into normal mice, streptozotocin-induced diabetic mice, and CY-induced neutropenic mice. These experimental models were developed to learn more about the

TABLE 5. *C. tropicalis* CFU data: colonization by organ[a]

Organ	Mean response ($\log_{10}$ CFU ± SEM[b])				
	Day 1	Day 2	Day 5	Day 7	Day 9
Kidney	3.07 ± 0.09	3.67 ± 0.16	5.45 ± 0.31	5.50 ± 0.29	5.12 ± 0.39
Liver	3.04 ± 0.10	2.77 ± 0.06	2.92 ± 0.29	2.40 ± 0.31	2.29 ± 0.33
Spleen	2.68 ± 0.14	2.54 ± 0.10	2.35 ± 0.27	1.92 ± 0.26	1.55 ± 0.22

[a] The response variable is $\log_{10}$ CFU. Responses have been averaged over strains and treatment groups.
[b] Standard errors of the relevant means. These standard errors do not reflect the correlation among means for the three organs.

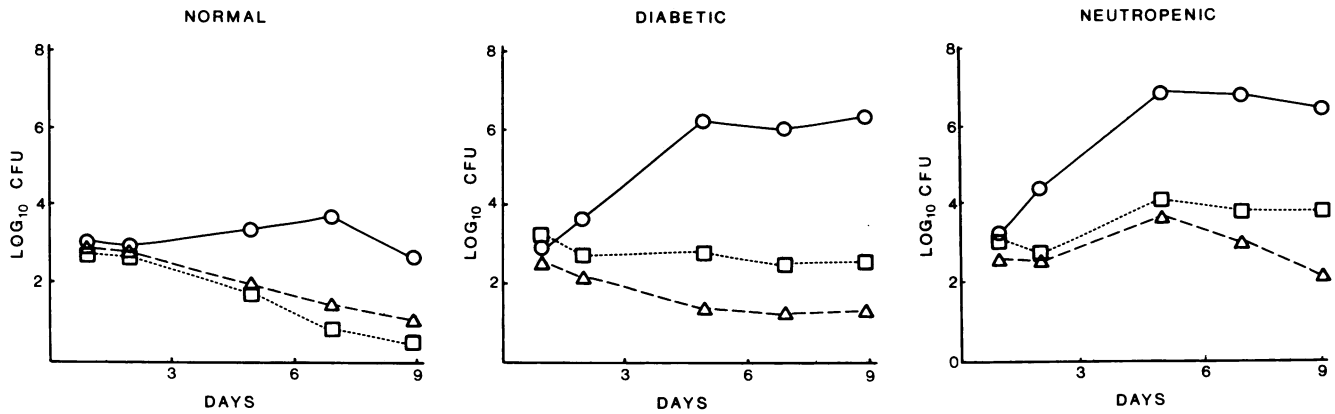

FIG. 2. CFU of *C. tropicalis* obtained from the liver, spleen, and kidneys of normal, diabetic, and neutropenic mice. The data are organized and illustrated for each organ. Symbols: ○, kidney; □, liver, △, spleen.

virulence and pathogenicity of this opportunistic yeastlike organism and to answer three questions: (i) are there differences among three strains of *C. tropicalis* in their lethality and virulence; (ii) are there differences in infection among normal, diabetic, and neutropenic mice; and (iii) is there a difference in colonization of *C. tropicalis* among major organs?

Our study demonstrated that one strain of *C. tropicalis* was more virulent for normal, diabetic, and neutropenic mice than the other two strains examined. This observation was based on lethality studies as well as determination of CFU of yeasts in the kidneys, liver, and spleen. The other two strains of *C. tropicalis* were comparable to each other in both lethality and colonization of major organs.

Our study determined that the order of group susceptibility to infection by each of the three strains of *C. tropicalis* was neutropenic > diabetic > normal. This result was not surprising considering the established increase in susceptibility of both diabetic and compromised human patients to opportunistic infections in general. However, it was of interest to verify this hypothesis in the experimental models examined in this study.

Our study also determined that the order of the extent of colonization of major organs for all three fungal strains and experimental groups was kidney > liver > spleen. The highest infection levels occurred in the kidney, indicating that the kidney is the target organ for experimental, systemic *C. tropicalis* infection. Although CFU fluctuated over time, from day 5 p.i., the data were consistent with the rank of kidney > liver > spleen.

In comparison with studies by other investigators, our study supports the observations by Bistoni et al. (2) of increased pathogenicity of *C. tropicalis* strains in CY-treated mice. Our study goes further by determining the pathogenicity of *C. tropicalis* in streptozotocin-induced diabetic mice as well. Our study also complements reports by Vecchiarelli et al. (15) and by Wingard et al. (18–20) who examined *Candida* yeast cell-murine immune cell interactions in vitro (15) and the pathogenicity of *C. albicans* and *C. tropicalis* in compromised patients (20) and in models of experimentally compromised animals (18, 19).

Thus, the data of Bistoni et al. (2), Wingard et al. (18–20), and Vecchiarelli et al. (15), as well as our own data, demonstrate the pathogenic potential of *C. tropicalis* to cause opportunistic infections. This information is important to the medical community, particularly to hospitals and clinics that are responsible for the treatment of compromised patient populations.

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