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Colonization of the Intestinal Tract of Conventional Mice with *Candida albicans* and Treatment with Antifungal Agents

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Conventional mice inoculated with *Candida albicans* per os were unable to maintain this organism in the intestinal tract as judged by decreasing numbers of yeast recoverable from feces. After inoculation with 10^7 cells/mouse, fecal counts ranged from 10^5 cells per g of feces to 5×10^3 cells per g of feces during a 12-day experimental period. Addition of various antibiotics to the drinking water did not result in any improvement in maintenance or stability of the gut population. A combination of X irradiation and administration of tobramycin or gentamicin, however, resulted in a stable population of *C. albicans* in the intestinal tract, with cell counts in the feces remaining constant at a level of about 10^6/g of feces for a period of 10 to 15 days. The usefulness of this model in assessing the effect of experimental drugs on *C. albicans* infections of the gut was demonstrated by the fact that treatment with a new antifungal antibiotic (A9145), amphotericin B, 5-fluorocytosine, or nystatin resulted in a reduction in the fecal counts of *C. albicans* from experimentally infected animals.

Attempts to establish an experimental *Candida* infection in the intestinal tract of conventional laboratory animals in order to study either the pathology of such an infection or the effectiveness of antifungal agents have not produced a satisfactory experimental model. It is well established that germ free mice and chickens can be colonized (1, 2, 4, 5) by *C. albicans*. However, *C. albicans* could not compete (4) with either the normal bacterial population in the gut or with *Escherichia coli* when both organisms were inoculated into germfree mice. Attempts to establish a stable population by the use of various antibiotics, associated with increased incidence of candidiasis in man, have not been successful or have shown variable results (2, 6). This study was undertaken to develop an experimental model to quantitate the effects of antifungal antibiotics on an intestinal infection caused by *C. albicans*. The requirements for such a model system were: (i) conventional mice be used; (ii) stable yeast populations be established; and (iii) known antifungal antibiotics show effectiveness as judged by a reduction of cell counts in the feces. These criteria have been met by a combination of X-ray treatment and administration of tobramycin or gentamicin.

MATERIALS AND METHODS

C. albicans, strain A26, a clinical isolate that we have shown to be virulent for mice when injected intravenously (3), was used for all experiments. An inoculum was prepared from a 24-h culture on Sabouraud dextrose agar by suspending the cells in saline and adjusting the optical density to 0.8 (620 nm), which corresponds to a cell concentration of 10^8 cells/ml. The intestinal infection was established by inoculating each animal by oral gavage with 0.1 ml of this yeast suspension. Twenty-four hours before inoculation, the animals were X irradiated with a sublethal dose of 400 R, administered at the rate of 50 R/min.

The effect of antibiotics on the stability of the infection was examined by including the compound in the drinking water or by administering the compound by gavage. The dose rate for antibiotics added to the drinking water was based on a 24-h water consumption of 5 ml/mouse. Mice were treated 10/cage, and the course of yeast growth in the intestinal tract was followed by making plate counts of feces (approximately 0.1 g/sample) collected daily from each cage. The fecal samples were suspended in 3 ml of saline, ground in a glass tissue homogenizer, and plated on Sabouraud dextrose medium containing 50 μg of chloramphenicol per ml. Colonies of *C. albicans* were counted after 48 h of incubation at 30 C.

The distribution of *Candida* in the gastrointestinal tract was judged from samples taken from animals 12 days postinfection. The animals were sacrificed, and the stomachs and intestines were removed and cut into sections. Each section was then ground in a glass tissue homogenizer with 4 ml of saline, and plate counts were made from aliquots of this extract.

Amphotericin B and nystatin were obtained from

E. R. Squibb, clotrimazole was obtained from Delbay Pharmaceuticals, and 5-fluorocytosine was obtained from Hoffmann-LaRoche. Tobramycin (Nebcin) and the experimental antifungal agent A9145 were obtained from the Lilly Research Laboratories. Gentamicin (Garamycin) is a product of Schering Corp. The minimum inhibitory concentration of these compounds for *C. albicans* (strain A26) was determined in an agar dilution assay using yeast-nitrogen base (Difco).

RESULTS

C. albicans can be maintained in the intestinal tract of orally infected mice and quantitated by plating the feces of these animals. In untreated conventional mice, however, the gut yeast population tends to drop rather rapidly after infection and shows a large day-to-day variation. Previous work in this laboratory (3) showed that X irradiation reduced the variation in the death pattern of a systemic *Candida* infection in mice. This immune suppressive procedure was examined to determine whether a similar effect could be found with an intestinal infection. Figure 1 shows a comparison of infected mice with and without X irradiation of 400 R 24 h before oral inoculation. Under both conditions, the yeast population was erratic after day 7, although animals that received the irradiation had consistently higher yeast counts. Because of the previous demonstration (4) that germfree mice were capable of maintaining a population of *C. albicans* in the intestinal tract and the fact that certain antibacterial antibiotics have been associated with *C. albicans* infections, a number of these antibiotics in combination with irradiation were examined for their effect on fecal counts after infection with *C. albicans*.

Figure 2 shows fecal counts from animals receiving vancomycin, kanamycin, and a combination of vancomycin plus kanamycin. Vancomycin treatment resulted in fecal cell counts essentially identical to control counts. The fecal cell counts in the presence of kanamycin alone or in combination with vancomycin were initially higher than controls; however, all conditions shown were quite variable and not sufficiently stable for a reliable experimental system. In addition, irradiated animals treated with vancomycin or kanamycin but not infected with *Candida* developed a fatal systemic infection apparently caused by a gram-negative rod, tentatively identified as an enterobacter. The same bacterium was found in animals orally infected with *C. albicans*, although those animals survived. Susceptibility testing showed this bacterium to be susceptible to tobramycin, gentamicin, and polymyxin B and resistant to

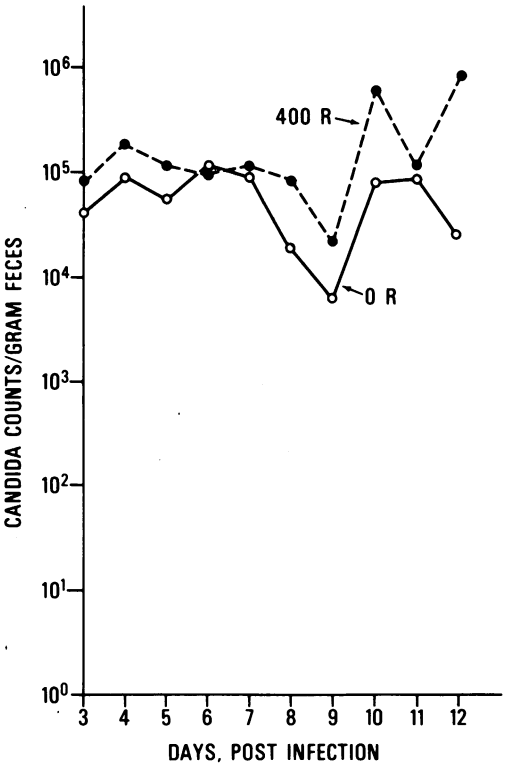

FIG. 1. *Effect of X irradiation on the gut Candida population.*

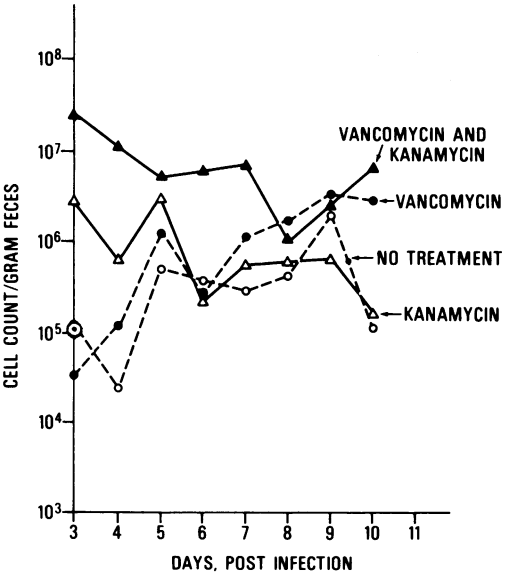

FIG. 2. *Effect of vancomycin (50 mg/kg per day), kanamycin (50 mg/kg per day), and vancomycin plus kanamycin (both at 50 mg/kg per day) on fecal Candida counts in mice X irradiated with 400 R. Vancomycin and kanamycin were administered by gavage.*

ampicillin, penicillin, carbenicillin, cephalothin, chloramphenicol, kanamycin, tetracycline, and vancomycin. Since the erratic *Candida* counts may have been associated with the growth of this bacterium, experiments were conducted to examine the effect of those antibiotics to which this organism was susceptible, i.e., tobramycin and gentamicin.

Figure 3 shows the effect of tobramycin and gentamicin (25 mg/kg per day), in combination with X irradiation, on fecal *Candida* counts. Administration of either of these antibiotics resulted in fecal counts between 10^6 and 10^5 cells per g of feces for a period of 13 days. The counts were quite stable, and these results have been repeated in many subsequent control groups with little change in variability. We selected to use tobramycin in our work with this model, although both antibiotics are equivalent in this system and give stable reproducible counts. Tetracycline treatment was also examined, but failed to enhance the stability of the gut yeast population.

During the first 3 days postinfection, considerable variation occurred in the fecal counts under almost all conditions. Therefore, in experiments to evaluate antifungal compounds, administration of the experimental compound and collection of fecal counts were not begun until the 3rd day after infection. To establish the incidence of infection in these animals after oral inoculation, the colon and rectum of untreated mice were removed at various times after inoculation and homogenized, and plate counts of the homogenate were made. Fifty mice were inoculated and placed 10 mice per cage. On days 3, 5, 7, 9, and 12, all of the animals in a single cage were sampled. Table 1 shows the average colony counts per animal and the number of animals in each cage from which *Candida* was recovered. The average counts per infected mouse varied by less than 10-fold during the experiment, and the number of uninfected animals per group was 1 or 2 except on day 12, where three animals were negative.

Earlier work (4) using germfree mice inoculated with *Candida* established that the yeast could be recovered from all parts of the intestinal tract. Results from a similar examination with mice treated with X rays and tobramycin are shown in Fig. 4. *Candida* was found throughout the intestinal tract, with highest numbers occurring in the stomach and cecum. Mice given 10^7 *Candida* cells were shown to have an established yeast population after plating 12 daily fecal samplings. Gut sections, including stomach, duodenum, and sections of

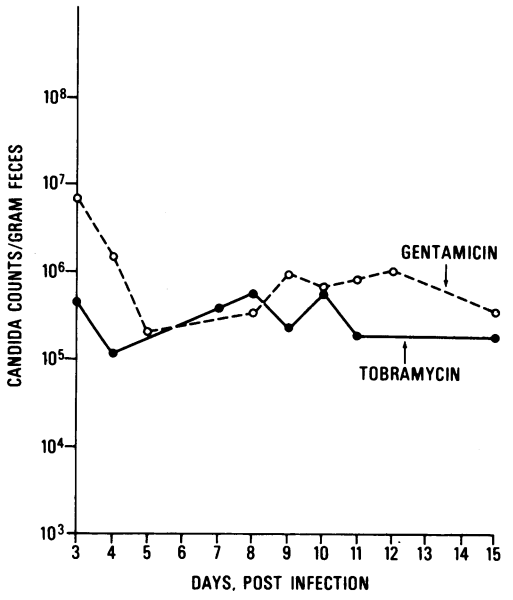

FIG. 3. *Effect of tobramycin (25 mg/kg per day) and gentamicin (25 mg/kg per day) on fecal Candida counts in mice X irradiated with 400 R. Tobramycin and gentamicin were added to the drinking water at a concentration of 0.09 mg/ml.*

TABLE 1. *Incidence of infection in mice after oral inoculation with C. albicans*

Days postinoculation	Avg colony count/infected animal (colon-rectum)	No. of animals infected/no. inoculated
3	3.66×10^5	10/10
5	1.23×10^5	9/10
7	8.73×10^4	8/10
9	5.37×10^4	9/10
12	3.19×10^5	7/10

ileum, cecum, colon, and rectum, were homogenized and plated for yeast cell counts. The average yeast cell counts of each section from four mice are summarized in Fig. 4. These yeast populations were quite consistent from animal to animal. After a drop in counts in the section just below the stomach, subsequent sections showed increasing counts which leveled off at the cecum, colon, and rectum. This shows that not only is the entire intestine colonized, but the stomach and cecum also harbor the yeasts. An attempt to assess the degree of infection in these animals versus colonization was made by microscopic examination of sections from various parts of the intestine. Because of moderate damage to the gut epithelium as a result of X irradiation, no meaningful assessment of inflammatory response due to the yeast could be

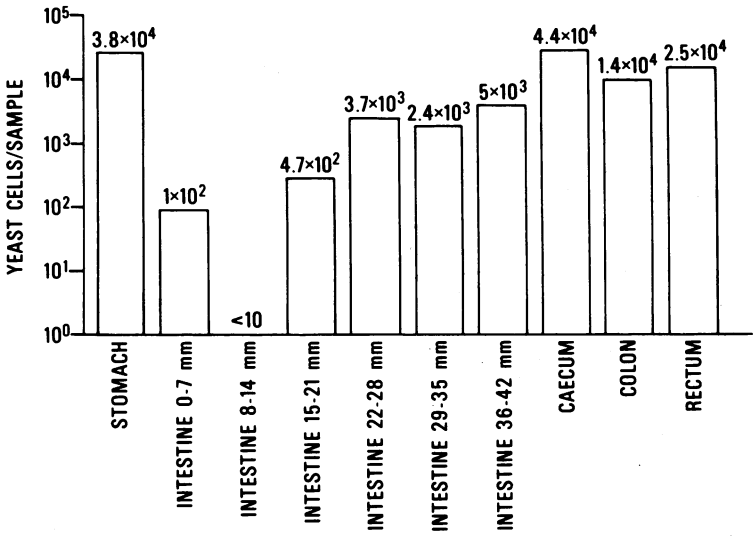

FIG. 4. *Distribution of Candida in the gastrointestinal tract of mice 12 days after oral inoculation with C. albicans. Mice were X irradiated with 400 R, and tobramycin was added to the drinking water at a concentration of 0.09 μg/ml.*

made. The fungus was present in all sections examined. In the unirradiated animals, only the yeast form was observed, and it was associated with the epithelial layer. The mycelial form, along with the yeast form, was found associated with the epithelium in X ray-treated animals. The presence of the mycelial form suggests an infection (6) in the X ray-treated animals as opposed to colonization as seen in the nonirradiated group.

The importance of the bacterial flora of the gut in competing with *C. albicans* has been well established by the use of germfree animals (4–6). The apparent competition between bacterial populations and *Candida* in normal mice may explain the inability of yeast to successfully colonize the gut of these animals. Since the animals used in this study were treated with X rays and tobramycin, the possibility exists that the bacterial population had been lowered and thus the competition normally observed with *Candida* was sufficiently reduced to allow proliferation of the yeast. Examination of the fecal flora of these animals indicated no apparent antibiotic-induced shift in the qualitative nature of the bacterial population, except for elimination of a virulent *Enterobacter* sp. Further, plate counts of these samples showed total bacterial counts approximately equivalent to those of control animals. Therefore, in this model infection, no evidence was obtained suggesting that a drastic shift in the bacterial flora or a reduction in the total number of bacteria could account for the establishment of *Candida* in the intestinal tract.

The usefulness of this system to assess the effectiveness of antifungal antibiotics on an intestinal infection as judged by a reduction in fecal counts is shown in Fig. 5. This experiment shows the effect of amphotericin B at 20 mg/kg, nystatin at 20 mg/kg, 5-fluorocytosine at 50 mg/kg, and a new antifungal antibiotic, A9145, at 10 mg/kg. Treatment with these compounds commenced 3 days postinfection. Amphotericin B and nystatin were administered by gavage, whereas 5-fluorocytosine and A9145 were administered in the drinking water. The effects of amphotericin B and nystatin were about equivalent, with cell counts dropping from 6×10^5 to around 5×10^4 cells/g after 5 days of treatment. 5-Fluorocytosine was slightly more effective, with counts dropping to 1×10^4 in 5 days and then leveling off. Of these four compounds, the experimental compound A9145 was the most effective in reducing cell counts, since the 5-day count was down to 5×10^2/g of feces and no *Candida* was recoverable after day 10.

We have examined other experimental antifungal compounds for an effect on this system; however, none have shown as dramatic an effect as A9145. Since the yeasts are distributed throughout the entire intestinal tract including the stomach, active compounds must be stable to pH changes and metabolism by the bacterial flora of the intestine. This may be a contributing factor to the activity of A9145, since a large

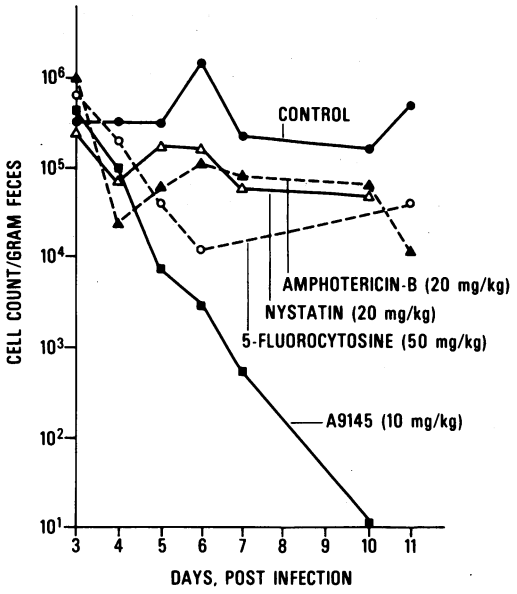

FIG. 5. *Comparison of antifungal antibiotics for the ability to reduce fecal yeast counts of Candida. Amphotericin (minimal inhibitory concentration [MIC] = 0.24 μg/ml) and nystatin (MIC = 0.94 μg/ml) were administered by gavage once each day. Compound A9145 (MIC = 0.47 μg/ml) and 5-fluorocytosine (MIC = 0.12 μg/ml) were administered in the drinking water. Mice were X irradiated with 400 R and received tobramycin in the drinking water at a concentration of 0.09 μg/ml.*

portion of the orally administered dose can be recovered from the intestinal tract.

DISCUSSION

Because of the importance of understanding the processes by which a relatively inefficient pathogen like *C. albicans* gains a position allowing it to cause infection, a number of attempts have been made to define a model system with which to study such infections. Systemic infection of mice of varying degrees of severity can be easily established depending on the inoculum size, the route of infection, or the immune state of the animals. However, the intestinal tract of mice appears to be a relatively poor environment for supporting the proliferation of this organism and as a reservoir for systemic infection. This may be a result of competition from the normal bacterial flora or because of an inability of *Candida* to penetrate the mucosal epithelium of the gut wall. The mechanism of this resistance to infection is not fully known. However, it is well documented that *Candida* is not a normal inhabitant of the mouse as it is in man, and severe measures must be used in order to establish an infection or colonization of the intestinal tract of these animals.

The system described here uses X irradiation at a relatively high, although sublethal, dose. No doubt a drastic change in the immune competence occurs in these animals as well as damage to the intestinal wall lining that may contribute to the resultant susceptibility to infection. Furthermore, to maintain a stable population in the gut, continuous administration of an antibiotic, tobramycin, is needed. Since previous work has indicated that *Candida* cannot compete with *E. coli* in the gut (4), the requirement for a broad-spectrum antibiotic possibly is involved in reducing the growth of gram-negative species. Certainly, the ability of microorganisms to survive in the intestine involves a complex set of competitive pressures. The effectiveness of a given antibiotic to stabilize *Candida* in the mouse gut may, however, involve selective reduction of a few bacterial species rather than general clearance of the bacterial flora. The fact that we could not detect any gross changes in the flora and that elimination of a specific *Enterobacter* resulted in stability of the yeast population suggests such a selective effect.

Attempts to determine whether the yeast had infected the epithelium of the gut were hampered by X irradiation damage. We did not look for evidence of sublethal infection as judged by the presence of yeast in body organs. However, there were no deaths among these irradiated animals attributable to *Candida* infection even though they are quite susceptible to systemic infection by the strain of *C. albicans* used. Therefore, during the test period of 10 to 13 days, there were probably few if any organisms escaping the gut, and systemic infection from this source seems not to occur as easily in mice as it apparently does in man (6).

The most interesting aspect of this system appears to be its possible usefulness for evaluating antifungal antibiotics. Since the fecal counts are quite stable and are reduced to various degrees by known and experimental antifungal compounds, this model may have predictive capabilities for detecting activity against this type infection as it occurs in man.

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