Systemic and Gastrointestinal Candidiasis of Infant Mice
After Intragastric Challenge

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Systemic and gastrointestinal infection can be established in infant mice after intragastric challenge with Candida albicans. Differences in virulence of the six strains tested were noted. As early as 3 h after infection, some but not all livers, spleens, and kidneys contained C. albicans, but the peak number of colony-forming units in these organs was seen at 6 h. The early colonization of the organs could not be attributed to aspiration of the inoculum since about 90% of lungs and livers tested yielded no colony-forming units at 10 to 15 min postinfection. In animals with systemic infections, lungs, livers, kidneys, and spleens showed similar numbers of colony-forming units within the organs during the first 6 h postinfection, and then the number declined progressively up to 72 h. The gastrointestinal tract was colonized throughout a 20-day period of study. Counts made at intervals beyond day 1 yielded between 106 and 108 colony-forming units in the stomach, ileum, and cecum. Preparatory techniques for scanning electron microscopy preserved the yeast, intestinal mucus layer, and epithelial surface and made it possible to visualize the association between the pathogen and host tissues within the digestive tract.

Generalized systemic infections produced by Candida albicans have increased in importance in recent years as causes of morbidity and mortality in patients undergoing chemotherapy for cancer (7, 17), immunosuppressive therapy (5), or prolonged antibiotic therapy (18). Passage through the gastrointestinal mucosa of such individuals is believed to be a likely portal of entry into the body since C. albicans has been shown capable of crossing human bowel walls (13). Studies of this type of behavior of C. albicans in experimental animals have been hampered, however, by lack of a suitable model.

Efforts to produce persistent infection with C. albicans in the digestive tracts of animals have been based on the use of adult conventional and germfree mice and of adult specific pathogen-free mice (14, 16). Since these approaches have not been fruitful, investigators have turned to procedures designed to compromise the animals. Turner et al. (19) found that mice became predisposed to infection with C. albicans per os after the animals had been treated with a combination of X-irradiation and tobramycin or gentamicin. The mice had well-colonized intestinal tracts for a period of 12 to 15 days. In other experiments, mice were made more susceptible to C. albicans infection after the administration of a combination of antibiotics and cortisone (4), antibiotics and cytostatic drugs (15), or a broad spectrum of immunosuppressive drugs. Umenai (21) was able to establish systemic infections in mice after oral administration of C. albicans only when the mice had been pretreated with a combination of antibiotics, X-irradiation, and dexamethasone.

Infant mice have been used successfully in several investigations of host-pathogen interactions and have certain advantages over other animals (1, 2, 9, 12, 20). In this paper, we describe the results of experiments with infant mice in which C. albicans, after intragastric challenge, established long-lasting intestinal colonization. These organisms also are shown to spread from the digestive tract in a large percentage of animals and create systemic infections.

MATERIALS AND METHODS

Animals. CFW mice obtained from Charles Rivers Farms, Wilmington, Mass., were used to establish a breeding colony in the departmental animal facilities. Offspring of these animals were used in all experiments.

Organism. C. albicans NS-33, the organism used for challenge, was obtained from G. D. Ahearn, Department of Biology, Georgia State University, Atlanta. Animal passage was carried out in adult female CFW mice inoculated in the lateral tail vein with a suspension of yeast which was subsequently isolated.
from kidney homogenates from moribund animals. Passage was repeated a second time. The culture finally used in these experiments was isolated from kidney homogenates from 5- to 6-day-old mice that had been infected by oral inoculation. Stock cultures were preserved by freezing at -70°C.

Other strains of _C. albicans_ used in these studies were sent by G. D. Ahearn, and additional strains were supplied by Edward Balish, Department of Microbiology, University of Wisconsin School of Medicine, Madison.

**Preparation of inoculum.** A subculture of the organism grown on Sabouraud dextrose agar for 24 h at 37°C was harvested with nonpyrogenic saline (Travenol; Travenol Laboratories, Inc., Deerfield, Ill.), washed twice by centrifugation, and resuspended to the estimated inoculum size. This estimate was based on repeated dilution counts of similarly prepared suspensions, whereas the actual challenge dose was determined by a dilution count at the time of challenge.

**Isolation of animals.** Infant mice 5 to 6 days old were removed from mothers 5 h before infection. The inoculum containing approximately $5 \times 10^8$ colony-forming units (CFU) in 0.05 ml was delivered intragastrically by using a 1-ml tuberculin syringe equipped with a blunted 21-gauge needle tipped with polyethylene tubing (Intramedic; Clay Adams, Div. of Becton, Dickinson & Co., Parsippany, N.J.). After infection, infants were returned to their mothers.

**Enumeration of organisms in tissues.** Mice were sacrificed by decapitation and dissected under aseptic conditions. Tissues were homogenized in 5.0 ml of sterile saline. Dilutions were plated on Sabouraud dextrose agar containing 50 µg of chloramphenicol per ml, and after a 48-h incubation at 37°C, the number of CFUs per gram (dry weight) of tissue was determined.

**Scanning electron microscopy.** Gastrointestinal tracts were removed at various times after infection, placed in chilled 0.1 M cacodylate buffer (pH 7.4), and cut into the following segments: stomach, upper intestine, midintestine, ileum, cecum, and large bowel. These segments were frozen in liquid nitrogen as described by Davis (3). The frozen tissue was cleaved with a chilled acetone-cleaned razor blade and placed in 2.5% glutaraldehyde in 0.1 M cacodylate buffer (pH 7.4) at 4°C. Samples were postfixed in 2% osmium tetroxide dissolved in the same buffer, dehydrated in an ethanol series, transferred through an amyl acetate series, and critical point dried by using liquid carbon dioxide. Specimens were coated with gold-palladium in a Denton DV-502 sputter coater equipped with an omniority stage. Samples were examined in an AMR 1900 scanning electron microscope at 30 kV and photographed by using Polaroid 55 P/N film.

**RESULTS**

**Virulence of _C. albicans_ for infant mice.** Six strains of _C. albicans_ were tested for virulence in intragastrically challenged infant mice (Table 1). Two strains (B311A and Southern) were weakly virulent and killed only a few animals at a challenge dose of $10^6$ to $10^7$ CFU/mouse. At a similar level of infection, the remaining strains (792B, CA9, CA 30, and NS-33) killed a larger percentage of animals (from 26 to 56%). Strain NS-33, the only one passed through mice by us before its use, was about as virulent as any tested and was satisfactory for additional studies.

**Systemic spread of infection to selected organs of individual mice.** After intragastric challenge with $10^7$ to $10^8$ CFU/mouse, animals were sacrificed at various times during a 72-h period postinoculation, and livers, kidneys, and spleens were scored for the presence or absence of _C. albicans_ (Table 2). Livers were almost uniformly infected at each of the three test periods employed during the first 24 h, and nearly 70% contained yeast at 72 h. The number of infected kidneys and spleens increased between 3 and 6 h postinfection but declined thereafter.

<table>
<thead>
<tr>
<th>Time post-inoculation (h)</th>
<th>B311A (1 x 10⁷)</th>
<th>Southern (2 x 10⁸)</th>
<th>792B (1 x 10⁸)</th>
<th>CA 9 (2 x 10⁹) to 1 x 10¹⁰</th>
<th>CA 30 (1 x 10⁸) to 3 x 10¹⁰</th>
<th>NS-33 (2 x 10⁹) to 6 x 10¹⁰</th>
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<tbody>
<tr>
<td>0</td>
<td>7/7</td>
<td>9/9</td>
<td>9/9</td>
<td>45/45</td>
<td>39/39</td>
<td>64/64</td>
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<td>9/9</td>
<td>9/9</td>
<td>42/45</td>
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<tr>
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<td>7/7</td>
<td>8/9</td>
<td>9/9</td>
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<td>32/39</td>
<td>45/60*</td>
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<tr>
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<td>6/7</td>
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<td>36/45</td>
<td>26/39</td>
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</tr>
<tr>
<td>72</td>
<td>5/6*</td>
<td>7/9</td>
<td>4/9</td>
<td>36/45</td>
<td>20/34*</td>
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</tr>
<tr>
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<td>5/6</td>
<td>7/9</td>
<td>4/9</td>
<td>32/43</td>
<td>19/34</td>
<td>32/60</td>
</tr>
</tbody>
</table>

*Strains B311A and 792 B were obtained from E. Balish. Strains Southern, CA 9, CA 30, and NS-33 were sent by D. G. Ahearn, and each was strain designation prefixed by Georgia State University in the designations he provided.

*b Numbers in parentheses are challenge doses expressed as CFU administered to each mouse.

*c One or more mice were sacrificed before the interval indicated, and body organs were plated to determine the number of _C. albicans_ cells present.
The peak contamination in the three organs was at 6 h. The rapidity with which these organs were invaded by C. albicans indicates that the yeast cells either made a prompt passage across the digestive tract wall or entered the circulation, possibly via the lymphatic drainage.

There is also the possibility that regurgitation and aspiration of the C. albicans suspension after or during the intragastric challenge provided a route of entry of the pathogen into the organs that, if unanticipated, would lead to an erroneous interpretation of the results. The volume of the inoculum was decreased from 0.1 to 0.05 ml as a result of some preliminary tests in which a solution of Evans blue was administered. With the smaller volume, it was rare that dye was discernible elsewhere than in the stomach and intestine. This afforded some assurance that the inoculum was not entering the lungs directly. In addition, when C. albicans was given in the usual manner, mice sacrificed from 10 to 15 min later were found to have no culturable yeasts in lungs and livers except for an occasional animal (1 in 9). Most likely then, C. albicans must be assumed capable of achieving systemic distribution after its introduction into the digestive tract by some as-yet-unexplained path. As evidence of this, each organ was homogenized, and 10-fold dilutions were prepared and cultured at the time intervals previously used (Table 3). At 3 and 6 h after inoculation, counts of C. albicans were within a less than fivefold range (log₁₀ difference of about 0.65, or 4.23 to 4.88 CFU/g) in all four organs. Counts in the liver declined only slightly at 24 and 72 h whereas by 72 h counts in the other organs were nearly 1 log unit lower than those at 6 h. As Table 1 shows, mice challenged with the NS-33 strain began to die after 12 h, and by 72 h most of those that were going to die were dead. It is not unlikely, therefore, that the smaller number of C. albicans found in organs at 72 h was, possibly, a result of a selection of mice capable of surviving infection.

Colonization of the gastrointestinal tract. Infant mice infected intragastrically with C. albicans showed persistent colonization in their gastrointestinal tracts, as evidenced by plate counts of homogenates of the different segments (Fig. 1). In most segments, a rapid drop occurred between 6 and 24 h, whereas counts in the stomach, ileum, and cecum plateaued or declined only slightly from day 1 through day 20. These segments were most heavily populated; the reasons for this are not yet understood.

**Scanning electron photomicrographs of the colonized digestive tract.** Scanning electron microscopy was employed to study the association between C. albicans and the gastrointestinal mucosa. Figure 2 is a scanning electron micrograph of an intestinal segment from an uninfected mouse. Plump villi can be seen, covered by a sheetlike mucus layer(s) which is largely intact. At 6 h after intragastric inoculation, there were many yeasts on the surface of

<table>
<thead>
<tr>
<th>TABLE 2. Body organs positive for C. albicans</th>
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<td>Time postchallenge (h)</td>
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<tr>
<td>-----------------------</td>
</tr>
<tr>
<td>3</td>
</tr>
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<td>6</td>
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<tr>
<td>24</td>
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<tr>
<td>72</td>
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* Numbers in parentheses are number positive/total number examined.

<table>
<thead>
<tr>
<th>TABLE 3. Organ counts from mice with systemic infections</th>
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<tr>
<td>Time post-inoculation (h)</td>
</tr>
<tr>
<td>----------------------------</td>
</tr>
<tr>
<td>3</td>
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<tr>
<td>6</td>
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<tr>
<td>24</td>
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<tr>
<td>72</td>
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</tbody>
</table>

* Log mean number of CFU ± standard error of the mean. The numbers in parentheses are numbers of body organs counted.
Fig. 2. Scanning electron micrograph of an intestinal section from an uninfected control mouse. An intact layer of mucus covers the villi. Bar = 50 μm.

Fig. 3. Scanning electron micrograph of an ileal section at 6 h after inoculation with C. albicans. Strands of mucus are indicated by arrows. Bar = 50 μm.

The villi (Fig. 3). Discontinuities in the mucus sheet (Fig. 3, arrows) and strands of mucus draped between villi are visible. Yeast are also seen attached to strands of mucus. Figure 4 shows yeast on the tip of an ileal villus. Several yeast cells appear to be entangled in mucus (Fig.
4, arrow). Many budding yeast cells are seen in the ileum at 6 h postchallenge (Fig. 5). In the same micrograph occasional yeast cells appear to be beneath the mucus layer (arrows).

DISCUSSION

To our knowledge, this is the first successful attempt to produce progressive lethal experimental candidiasis in laboratory animals in-
ected via the oral (gastrointestinal) route without some form of additional prior manipulation designed to compromise the host. The 5 h of fasting to which the infant mice were subjected just before challenge is a form of stress, but it was employed so as to permit the digestive tracts to become empty of food contents. The return of the animals to their mothers immediately after infection should have minimized changes elicited by lack of nourishment. The microorganisms administered to the mice quickly became systemic via distribution and also successfully colonized the digestive tract. The technique used for inoculation allows precise control over the challenge dose and maintains the natural barriers to colonization. These include gastric secretions, peristalsis, the mucus layer, and the indigenous flora of the germ-free tract. Moreover, infant mice have the advantage in following the kinetics of the infection because they do not have an indigenous yeast microflora that might confuse the detection of C. albicans.

The use of infant mice for oral challenge with Vibrio cholerae (9) has made it possible to evaluate several vaccines administered to female mice just before mating. The passive immunity the mothers confer on their offspring provides high levels of protection when the best vaccines are administered (10). It is likely that similar studies now in progress will permit the assessment of vaccines derived from C. albicans.

The infant mouse model of candidiasis also lends itself to a study of how compromising agents contribute to the severity of the infection. Extending the persistence of C. albicans to a time when the mice are reaching maturity should make it possible to study various aspects of active immunity in this disease. Of equal significance is the value of this model in screening and evaluating antifungal (antifungal) therapeutic agents.

F"citr. 1 microscopy should make it possible to study the sequence of events involved in colonization of the gastrointestinal tract by C. albicans, the nature of the association of the microorganisms with the mucosal surface, and the mechanism of penetration of the gut wall that accompanies the initiation of systemic spread. The techniques employed in this study preserve the mucus layer and the association of the yeast with the mucus layer and with the epithelial surface.

ACKNOWLEDGMENTS

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LITERATURE CITED


