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Experimental *Candida albicans* Infection in Conventional Mice and Germfree Rats

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Swiss-Webster white mice were intravenously infected with various doses of *Candida albicans*, and the viable units in their spleens, livers, lungs, and kidneys were determined at various intervals after challenge. The results showed that *C. albicans* multiplied to a greater extent in the kidneys of mice than in their spleens, lungs, or livers. The infection in mice was chronic; increasing numbers of *C. albicans* were observed in their kidneys until about 17 to 24 days postchallenge. Clearance of *C. albicans* from infected kidneys was not symmetrical, since the number of viable *C. albicans* in one kidney did not coincide with the viable counts observed in the opposite kidney of that same animal. Male and female mice did not differ in their overall susceptibility (50% lethal dose test) or in the number of viable *C. albicans* in the kidneys at various time intervals after infection. *C. albicans* also multiplied in the kidneys of germfree rats; however, the peak of the *C. albicans* infection in their kidneys occurred earlier than in those of conventional mice.

Candida albicans is a member of the normal flora of many mammals. The incidence of human disease caused by this organism has increased steadily in recent years due to the wider use of immunosuppressive drugs and broad-spectrum antibiotics (14). *Candida* disease is also a serious problem for burn patients, patients suffering from various neoplasms, and people with immunological defects (11, 14). The variety of predisposing factors, the range of clinical disease, and the poor antibiotic chemotherapy now available have made candidiasis a serious clinical problem. The need to understand the immunity, host-parasite interactions, and pathogenesis involved in human disease due to *C. albicans* has made it desirable to describe a reliable animal model for candidiasis that mimics the systemic disease seen in humans.

Several animal models for disseminated candidiasis have been described in the literature. Baine et al. (1) used the rabbit as a model for disseminated candidiasis and found that the liver and the lungs cleared the greatest amount of intravenously injected *C. albicans*. Hasenclever and Mitchell (3) and Hurley (5) studied disseminated candidiasis in mice and established that the infection is of a chronic nature. Hurley and Winner (6) described the histological picture of experimental *C. albicans* disease in the tissues of mice and claimed that there is a strong resemblance in the histological pattern of disease in human and murine disseminated candidiasis. In each of the latter studies, however, quantitation of the number of *C. albicans* viable units in the tissues of infected mice was not carried out.

Edwards et al. (2) have developed a rabbit model for experimental *C. albicans* endophthalmitis. Intravenous injections of *C. albicans* into rabbits resulted in a *C. albicans* infection of the chorioretina, kidneys, and other organs. Enumeration of the viable *C. albicans* in the chorioretina and the kidneys showed that the infection peaked in 10 to 20 days and was eliminated after 80 days. Hurley and Fauci (4) found that intravenous injections of *C. albicans* into guinea pigs resulted in a chronic infection of the kidneys and heart, whereas other internal organs cleared the *C. albicans* infection within 4 days postchallenge (4). Work done with the mouse model for disseminated candidiasis has shown that the kidneys are the most severely infected organs in this species as well (9). Enumeration of the viable units of *C. albicans* in mice shows that the infection in the kidneys does not appear to peak until about 24 days postchallenge (9).

Quantitation of the number of viable *C. albicans* in the tissues of experimentally infected animals was not done in many of the studies reported in the literature up to this time. Typical dependent variables in the latter studies have included gross and microscopic observations of infected tissues or the assessment of percent mortality of challenged animals. This

paper reports the results of studies designed to further characterize the course of an experimental *C. albicans* infection in the tissues of mice by quantitating the number of viable *C. albicans* present in the organs at various time intervals after intravenous challenge. Since conventional mice and other conventional animals may have *C. albicans* (or other yeasts) as a part of their flora, these animals could have been infected by the yeast prior to the time of experimental challenge. For this reason, we have also studied the course of an experimental *C. albicans* infection in germfree rats.

MATERIALS AND METHODS

Microbial organism. *C. albicans* strain B311 (type A) was originally obtained from H. F. Hasenclever (National Institutes of Health, Bethesda, Md.). It was maintained in our laboratory by monthly transfers on Sabouraud dextrose agar slants (GIBCO, Madison, Wis.) with 0.4% yeast extract (Difco Laboratories, Detroit, Mich.) and stored at −70 C in 0.3 ml of fresh Sabouraud dextrose broth.

The morphological and biochemical characteristics of the *C. albicans* B-311 strain were verified by microscopic observations, colonial morphology on Sabouraud dextrose agar, the formation of germ tubes in human serum, the formation of chlamydospores on chlamydospore agar (Difco), and by sugar fermentation reactions. The 45-day 50% lethal dose of this strain was found to be 1.4×10^4 viable units for male mice and 1.6×10^4 viable units for female mice (12).

Mice. All mice were conventional Swiss-Webster white males and females weighing 22 to 29 g (Rolfsmeyer, Madison, Wis.). The possibility of any intercurrent infections of the mice was eliminated prior to each study by sacrificing several animals, chosen at random, and plating homogenates of spleen, liver, lungs, and the kidneys on both 5% defibrinated sheep blood agar (Difco) and Sabouraud dextrose agar plates. All animals were housed according to a random-order scheme.

Germfree rats. Sprague-Dawley germfree rats, of both sexes, were used in this experiment. The ages of the animals varied between 4 and 6 months at the time of infection. All animals were bred and housed in flexible-film germfree isolators at the University of Wisconsin Germ-Free Laboratory and were fed a crude, pelleted, autoclaved L5010C diet (Ralston Purina Co., St. Louis, Mo.).

Challenge procedure. Saline dilutions of the stored *C. albicans* suspension were carried out to obtain the proper challenge dose. A 0.1-ml dose of the appropriate suspension was injected into the left lateral tail vein. Challenge of mice with *C. albicans* was always carried out according to a random-order scheme. Germfree rats were anesthetized with sodium pentobarbitol and restrained with a metal rat holder. Rats were injected by intracardiac puncture.

Enumeration of the *C. albicans* viable units. Mice were sacrificed by cervical dislocation and dissected immediately under aseptic conditions. Rats were sacrificed by a lethal exposure to ether. The appropriate organs were removed aseptically and placed in tissue homogenizers. Sterile saline was added so that the final volume in a homogenizer was 5.0 ml. Each tissue was homogenized in a glass tissue homogenizer, dilutions were made of the homogenate, and the number of *C. albicans* viable units in the homogenate was determined by the pour-plate dilution method using Sabouraud dextrose agar. Colonies on Sabouraud dextrose agar were counted after 48 h of incubation at 35 C and are expressed as the number of viable units in the organs ± the standard error. The Student *t* test was used to assess the significance of the results (16).

RESULTS

Course of an experimental systemic *C. albicans* infection in mice. Table 1 shows the clearance of *C. albicans* from the bloodstream by the lungs, spleen, liver, and kidneys 60 to 120 min after intravenous challenge with 5.0×10^5 or 4.25×10^3 viable units. The liver was capable of clearing the highest percentage of the injected *C. albicans* (Table 1). On a weight basis, however, the kidneys were the most efficient organs at clearing the organism from the bloodstream.

Figure 1 shows the data for the course of an

TABLE 1. *Distribution of C. albicans in the internal organs of female mice after intravenous injection*[a]

Challenge dose	Organ	$\log_{10}$ VU of *C. albicans*		$\log_{10}$ VU of *C. albicans*	
		Per organ	% Clearance[b]	Per g of tissue	% Clearance[c]
5.0×10^5	Liver	4.52	6.6	5.14	27.5
	Lungs	4.03	2.1	4.05	2.2
	Kidney	3.33	0.4	3.07	1.0
	Spleen	2.94	0.1	3.42	0.5
4.25×10^3	Liver	3.06	27.3	2.96	21.2
	Lungs	2.83	16.0	2.88	18.3
	Kidney	1.99	2.3	2.23	4.0
	Spleen	1.46	0.6	1.59	0.9

[a] Sixty to 120 min after challenge. VU, Viable units.
[b] Percent clearance = (VU per organ/VU injected) × 100.
[c] Percent clearance = (VU per gram of tissue/VU injected) × 100.

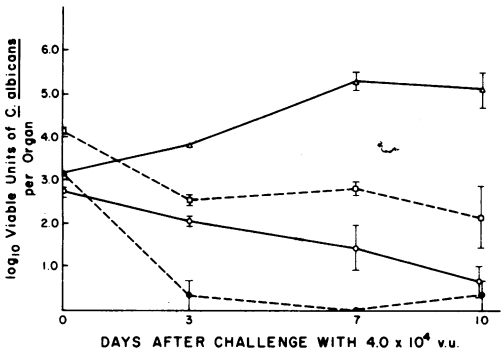

FIG. 1. *Course of an experimental C. albicans infection in the lungs (●), spleen (○), liver (□), and kidneys (△) of mice receiving a challenge dose of 4.0 × 10⁴ viable units (v.u.). Each point is the mean of three or five mice. Similar trends were observed when animals were challenged with 5 × 10⁵ or 4.25 × 10³ viable units of C. albicans.*

experimental *C. albicans* infection in the lungs, spleen, liver, and kidneys after an intravenous challenge with yeast cells. Although *C. albicans* persisted in each of these organs for the 10- to 14-day period of observation, it always multiplied to the greatest extent in the kidneys (Fig. 1).

Course of an experimental *C. albicans* infection in the kidneys of male and female mice. There did not appear to be a difference in the 50% lethal dose for male and female mice used in this study (1.4×10^4 for male mice and 1.6×10^4 for female mice). Other investigators have reported that females are more resistant than males to infections by several microorganisms (7, 10, 15, 17), including *C. albicans* (13). Microbial enumeration after intravenous challenge could be a more sensitive means of detecting sex differences in the susceptibility of mice to *C. albicans* than a 50% lethal dose study. For this reason, we decided to use microbial enumeration to assess the temporal course of an experimental *C. albicans* infection in the kidneys of male and female mice.

Equal numbers of male and female mice were challenged with 10^4 to 3×10^4 viable units intravenously. Animals were then sacrificed at various times after challenge, and the number of *C. albicans* viable units in each kidney was determined. The data from this experiment are presented in Fig. 2. The results show that there was no significant difference in the capacity of *C. albicans* to grow in the kidneys of male and female mice for the 24-day period of study. It is also apparent that *C. albicans* is not eliminated from the kidneys of male or female mice through day 24.

Comparison of the growth of *C. albicans* in the left and right kidneys of challenged mice. During these experiments with *C. albicans*-challenged mice, it became obvious that the gross appearance of one infected kidney did not always coincide with a gross appearance of infection in the opposite kidney of the same animal. Often, one kidney was edematous and very enlarged and the other kidney appeared normal. For this reason, a comparison of the number of viable units of *C. albicans* in each kidney was carried out with time after challenge.

Because the previous experiment indicated no difference in the overall growth of *C. albicans* in the kidneys of male and female mice, and because of the aggressive behavior of male mice housed in groups, only female mice were used in these experiments. Since the course of infection in the kidney is of a chronic nature, a sacrifice interval of 7 days was chosen. The infection of the kidneys appeared to peak at about day 17, so the first sacrifice was carried out 10 days after infection.

Female mice were challenged intravenously with 10^4 viable units of *C. albicans*. At each sacrifice interval, the kidneys were removed and homogenized separately. In this way the viable *C. albicans* in each kidney could be estimated for each animal. It can be seen in Fig. 3 that there was no significant difference in the numbers of viable *C. albicans* isolated from kidneys when all of the left kidneys were compared with all of the right kidneys.

Since our observations on the gross appearance of one infected kidney still did not always coincide with the appearance of infection of the opposite kidney, a further evaluation of the data was carried out. Figure 4 shows the results of a comparison between the kidney with the highest viable unit counts (right or left kidney)

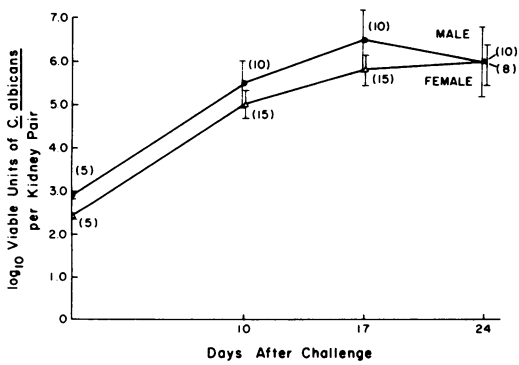

FIG. 2. *Course of an experimental C. albicans infection in the kidneys of male (●) and female (△) mice. The number of mice used to obtain each data point is given parenthetically.*

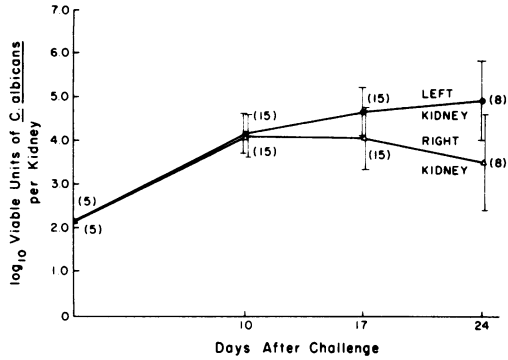

FIG. 3. *Course of an experimental C. albicans infection in the left (●) and right (△) kidneys of female mice. The number of mice used to obtain each data point is given parenthetically.*

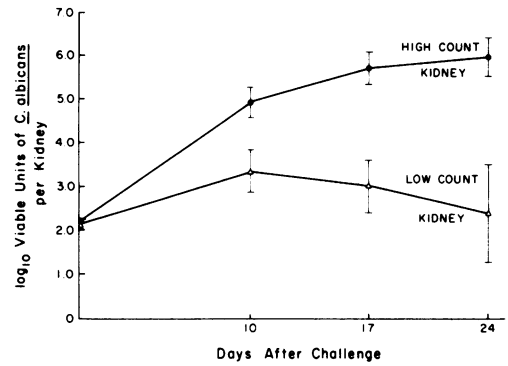

FIG. 4. *Asymmetrical nature of the course of an experimental C. albicans infection in the kidneys of female mice. Each data point is the mean of at least eight mice, except the points for day 0, which represent the mean of five mice. The kidney in each mouse with the higher number of C. albicans viable units is considered the "high count kidney" (●). The kidney with the lower number of viable units is considered the "low count kidney" (△). The bars represent the standard error of the mean.*

and the kidney, from the same animal, with the lower viable unit counts of *C. albicans* (left or right kidney). It can be seen in Fig. 4 that there is indeed a significant difference, with time after challenge, in the populations of *C. albicans* in the kidneys of a given mouse. To illustrate the latter point more clearly, results in Table 2 show the percentage of animals with at least a 2-log difference in the number of viable *C. albicans* in their kidneys with time after challenge. On day 10, 33% of the animals had at least a 2-log difference in the number of viable *C. albicans* in their kidneys. The other 67% of the mice on day 10 had kidneys with comparatively equal numbers of culturable *C. albicans*. On day 24 after challenge, 75% of the animals had at least a 2-log difference in the *C. albicans* viable unit counts in their kidneys, and only 25% of the mice manifested similar numbers of organisms in both kidneys. Also shown in Table 2 is the percentage of animals in which one kidney was free of any viable *C. albicans* and the opposite kidney was still heavily infected. On day 10, 20% of the animals had completely cleared the *C. albicans* in one kidney, whereas the other kidney still remained infected. At the termination of the experiment, the majority (75%) of the mice manifested a *C. albicans* infection in only one of their two kidneys.

Course of an experimental *C. albicans* infection in germfree rats. Due to the ubiquitous nature of *Candida* sp., it is possible that conventional mice may have already been infected with *C. albicans* prior to an experimental *C. albicans* challenge. It is unlikely, on the other hand, that the germfree animal has had any contact with viable yeasts and, as such, should be immunologically virgin as far as live *C. albicans* is concerned. There is some possibility, however, that the germfree animals have been exposed to antigens from dead *C. albicans* (or other yeasts) in their food. This study was carried out to determine if there is a difference in the course of an experimental *C. albicans* infection in the kidneys of an animal that has not been exposed to viable microorganisms.

Fourteen male and female germfree rats were injected with 1.1×10^4 viable units of *C. albicans*. The number of viable *C. albicans* per kidney was then determined at various times after challenge (Fig. 5). From these data it can be seen that *C. albicans* multiplied in the kidneys of the gnotobiotic animals. It appears, however, that the peak of the *C. albicans* involvement occurred at some time near day 10 postinfection, and the organism was slowly cleared from the kidneys after that time. After reaching a peak of about 10^6 viable units on day

TABLE 2. *Asymmetrical clearance of C. albicans from the kidneys of female mice*

Days post-challenge	% of mice with a higher number of *C. albicans* VU in one kidney than the other[a]	% of mice with no *C. albicans* VU in one kidney and at least 2 logs in the other
0	0	0
10	33	20
17	53	40
24	75	75

[a] A difference in the number of viable units (VU) is arbitrarily defined as 2 logs or more.

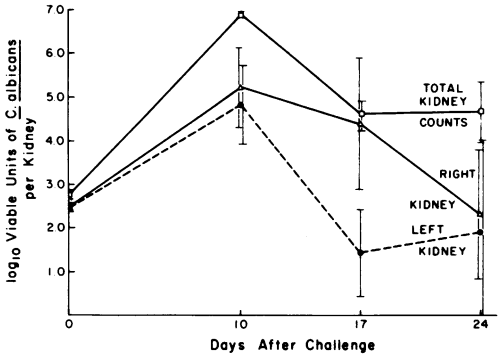

FIG. 5. *Course of an experimental C. albicans infection in the kidneys of germfree rats. Each data point represents the mean of at least three rats.*

10, the average number of viable units in the kidneys on day 17 was about 10^5 and decreased to about 10^4 on day 24.

A difference in the involvement of *C. albicans* between kidneys in each gnotobiotic rat was again evident. At each sacrifice interval, the number of viable *C. albicans* in the left kidneys was not signficantly different from the counts in the right kidneys. The renal infection of *C. albicans* in germfree rats was similar to the infection in conventional mice in the latter respect. The data in Table 3 show that clearance of the renal infection in germfree rats also appeared to be asymmetric, just as it was in conventional mice. Indeed, by the end of the experiment, at day 24 postinfection, 3/4 of the gnotobiotic rats had one kidney free of any viable *C. albicans*, whereas the opposite organ was still heavily infected.

DISCUSSION

The data reported here for mice show that the kidney is the most susceptible organ to a *C. albicans* challenge and is in agreement with data already reported in the literature (3, 5, 6, 8, 9, 18). We also observed that, although *C. albicans* persisted in the spleen, lungs, and liver for at least 13 days after challenge, it did not consistently multiply to any great extent in these organs. It is not clear why the kidneys are more susceptible to *C. albicans* than other organs. The number of resident phagocytes in the kidneys may be less than in other organs, such as the lungs and liver, or the kidneys may be a poor environment for phagocytic cells to kill *C. albicans* (8). The fact that the kidneys clear the greatest percentage of the inoculated *C. albicans* (i.e., on a weight basis) from the bloodstream may also contribute to their susceptibility and inability to control the growth of the pathogenic yeast.

There are several reports in the literature that males are more susceptible than females to disease from infections by a wide variety of microorganisms, including *C. albicans* (7, 10, 13, 15, 17). However, the 50% lethal dose data for this strain of *C. albicans* did not show a sex difference in the susceptibility of mice to *C. albicans*. Also, the temporal course of an experimental *C. albicans* infection in the kidneys of male mice, as studied by microbial enumeration, is not significantly different from the course of infection observed in the kidneys of female mice. Our results are contrary to the results reported by Rifkind and Frey (13). The latter investigators found that female mice were more resistant than male mice to a *C. albicans* infection. The experimental conditions of the latter workers differed from those used in our study with respect to both the dependent and independent variables. Rifkind and Frey found that the percentage of challenged mice with urine cultures positive for *C. albicans* was greater for males than for females (13). Similarly, the percentage of mice with a positive kidney culture, 10 weeks after challenge with *C. albicans*, was also greater for males than for females (13). In the latter study, however, quantitation of the viable *C. albicans* in the kidneys was not carried out. In addition, both the challenge dose and the route of injection of the *C. albicans* were unlike those used in our experiments. It may be argued that the experimental conditions used in our studies made it impossible to detect a greater susceptibility of males to challenge with *C. albicans*. If males are more susceptible than females to disease from *C. albicans*, then one would expect that the number of colony-forming units of *C. albicans* in the kidneys should differ for female and male mice with time after challenge. Our

TABLE 3. *Asymmetrical clearance of C. albicans from the kidneys of germfree rats*

Days post-challenge	Fraction of rats with a higher number of *C. albicans* VU in one kidney than in the other[a]	Fraction of rats with no *C. albicans* VU in one kidney and at least 2 logs in the other
0	0/4	0/4
10	2/3	0/3
17	2/3	2/3
24	3/4	3/4

[a] A difference in the number of viable units (VU) is arbitrarily defined as 2 logs of *C. albicans* VU or more.

data indicate that the host factors responsible for resistance to *C. albicans* are relatively equal (at least for the first 24 days after challenge) in male and female mice.

A very interesting aspect of the mouse renal candidiasis model was revealed when the number of viable *C. albicans* in one kidney was compared, at various times after challenge, with the number in the opposite organ of the same animal. The number of colony-forming units of *C. albicans* was not the same for both kidneys of most experimental animals by 24 days after infection. Both kidneys appeared to receive an equal number of yeast cells after intravenous challenge, but for some reason one kidney was able to eliminate the *C. albicans* and the opposite organ was not. To our knowledge, this phenomenon has not been previously reported in the literature. The mechanisms that function to eliminate *C. albicans* from only one of the two kidneys are not understood at this time. A physiological difference between the left and right kidney could account for this difference in clearance. This physiological difference could possibly be associated with a greater blood flow to one of the two kidneys, greater urine flow from one of the kidneys, or some anatomical difference between the kidneys. It seems unlikely, however, that these factors alone could be responsible for the asymmetrical susceptibility of the kidneys.

C. albicans also appears to be able to multiply in the kidneys of germfree rats. Although the kidneys were the only rat organs cultured in these experiments, it appeared from gross observations at autopsy that the kidneys were the most diseased organs in this species also. The renal infection by *C. albicans* does not appear to be as prolonged in the gnotobiotic rat as it is in the conventional mouse. The number of *C. albicans* viable units in the kidneys of challenged germfree rats appeared to peak on day 10 postinfection. The peak in the number of colony-forming units of *C. albicans* in conventional mice was between days 17 and 24. From results reported in the literature, it appears that an experimental renal infection by *C. albicans* is more chronic in mice than in rabbits or guinea pigs (2, 4, 18). Therefore, the shortened infection is not unique to germfree rats.

Just as in conventional mice, the kidneys of a given infected gnotobiotic rat do not appear to be equally susceptible to *C. albicans*. This indicates that the immune mechanisms responsible for clearance of the *C. albicans* infection from one kidney in preference to the other kidney are not peculiar to mice. It also indicates that prior exposure to viable *C. albicans* is not necessary for the asymmetrical clearance to occur. And, finally, it shows that prior damage to only one kidney of the animal by a previous bacterial infection is likewise not required for the appearance of this phenomenon of asymmetrical clearance of *C. albicans* from the kidneys.

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