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Organ Localization in Mice Challenged with a Typical *Candida albicans* Strain and a Pseudohyphal Variant[1] (39780)

ZOE A. EVANS[2] AND D. N. MARDON

Department of Microbiology, Virginia Commonwealth University, Richmond, Virginia 23298, and Department of Biological Sciences, Eastern Kentucky University, Richmond, Kentucky 40475

Candida albicans is an opportunistic pathogen. This dimorphic fungus grows in both a yeast-like and a pseudohyphal form. The relative pathogenicity of these two morphological forms has long been a subject of concern since both yeast-like and pseudohyphal elements are commonly found in tissues infected with *C. albicans* (1, 2). However, recent investigations have shown that the lethal response in mice and rabbits is more severe with yeast-like cells of this microorganism than with pseudohyphal preparations (3, 4). Because the phagocytic response of the reticuloendothelial system (RES) is an important component of host resistance to disseminating microbes, this study was undertaken to determine whether the differential virulence of the two forms of *C. albicans* is associated with different localization patterns in the major RES organs of mice.

Materials and methods. All animals used in this study were preleukemic male AKR mice obtained from the Jackson Laboratories, Bar Harbor, Maine. They were allowed to adjust to their new environment for 1 week prior to experimentation and were 6–8 weeks old at the start of each experiment. The mice were fed lab chow and water *ad libitum*.

The two strains of *C. albicans* used throughout this investigation were ATCC 10261, which grows as typical yeast-like cells and is virulent in mice, and ATCC 10259-R, a pseudohyphal variant of ATCC 10261 which is of low virulence in mice. Radioactive *C. albicans* cells were prepared by aseptically transferring cells from a 24-hr-old stock slant (Sabouraud dextrose agar, BBL) to a minimal glucose-salts-biotin broth (3) containing 0.2% glucose and supplemented with 50 μCi of D-[U-^{14}C]glucose/ml of medium. After growth in this medium at 27° for 18 hr in a New Brunswick gyrorotary water-bath shaker, the resultant cultures were harvested, washed, and suspended in sterile 0.85% saline. All suspensions were adjusted to the same predetermined turbidity (35 Klett units) using a Klett-Summerson colorimeter, then diluted 1:4 with sterile saline. Each mouse was injected via a lateral tail vein with 0.5 ml of the appropriate cell suspension. Although all standard doses contained the same cellular mass, those of ATCC 10261 contained an average of 9×10^5 yeast-like colony forming units (CFU) while each dose of ATCC 10259-R contained approximately 4×10^5 pseudohyphal CFU.

Organ localization assays were done at various time intervals after mice were injected with either yeast-like or pseudohyphal preparations. Replicate blood samples (0.02 ml) were collected in heparinized micropipets from the retro-orbital plexus and hemolyzed in 0.2 ml of sterile distilled water. The mice were then sacrificed by cervical dislocation and liver, lungs, spleen, and kidneys were excised. Each organ was homogenized in 2.0 ml of sterile distilled water using Ten-Broeck homogenizers. Radioactivity was measured by placing the hemolyzed blood samples or 0.2-ml samples of each tissue homogenate in scintillation vials containing 10 ml of Triton X-100 plus toluene–Liquifluor, then counted in a Beckman LS-355 liquid scintillation spectrometer. Viable *Candida albicans* units were determined by spreading hemolyzed blood samples and serial dilutions of the organ homog-

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[2] Send reprint requests to Zoe A. Evans, Department of Microbiology, Medical College of Virginia, Box 847, Virginia Commonwealth University, Richmond, Va. 23298.

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enates on Mycosel agar (BBL) plates and counting the colonies which appeared after incubation of the inoculated plates at 37° for 48 hr. Radioactive and viable unit data were normalized to indicate percentage of the original inoculum in each organ.

Results. Mean survival time of animals injected with a standard inoculum of *C. albicans* ATCC 10261 was 4.8 days but all animals given a standard dose of *C. albicans* ATCC 10259-R lived beyond the 30-day observation period. To obtain comparable rates of killing with both strains of *C. albicans* it was necessary to use a dose of strain 10259-R containing more than 40 times the number of organisms required with strain 10261.

More than 90% of the radioactive cells were cleared from the blood within 30 min after injection of either *Candida* strain; however, organ localization patterns of these two morphological forms differed. Mice inoculated with the pseudohyphal variant (ATCC 10259-R) showed a greater initial localization of radioactive organisms in the lungs than did mice receiving the yeast-like strain (Fig. 1). Very low percentages of viable units or radioactive counts were detected in the lungs within 12 hr after inoculation of either strain.

When the liver was examined for localization of the organisms, a pattern was observed which was opposite to that found in the lungs. Within 15 min after injection a higher percentage of the original inoculum accumulated in the liver when the yeast-like strain was injected than when the inoculum was pseudohyphal (Fig. 2). In addition, relatively greater numbers of viable units of both *Candida* strains remained in the liver between 12 and 96 hr after inoculation than were detected in the lungs during the same period.

Radioactive and viable unit data indicate that low percentages of *Candida* organisms localized initially in the kidneys (Fig. 3A). However, organisms began to multiply in the kidneys within 24 hr after injection of the yeast-like inoculum, as shown by increasing percentages of viable units in the kidneys. Figure 3A also shows that growth did not occur within 96 hr following injection of the pseudohyphal variant.

The spleen accumulated relatively few organisms of either strain (Fig. 3B). Less than 10% of the original inoculum was found in the spleen at each time tested, indicating that this organ may not be of primary importance in clearing either yeast-like or pseudohyphal elements from the blood.

Calculation of radioactivity, in terms of counts per minute per milligram of tissue, showed that on a weight basis lung tissue is more efficient than liver tissue in clearing the blood of yeast-like as well as pseudohyphal elements (Table I). Also shown in Ta-

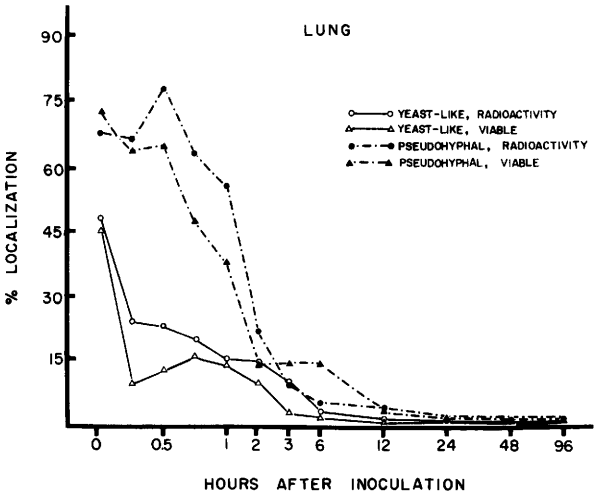

FIG. 1. Localization of radioactivity and viable units in the lungs of AKR mice inoculated iv with 0.5 ml of a radioactive yeast-like (ATCC 10261) or pseudohyphal (ATCC 10259-R) suspension of *Candida albicans*. Each point represents the mean for three animals. SE ranges from ±0.2 to ±19.5%.

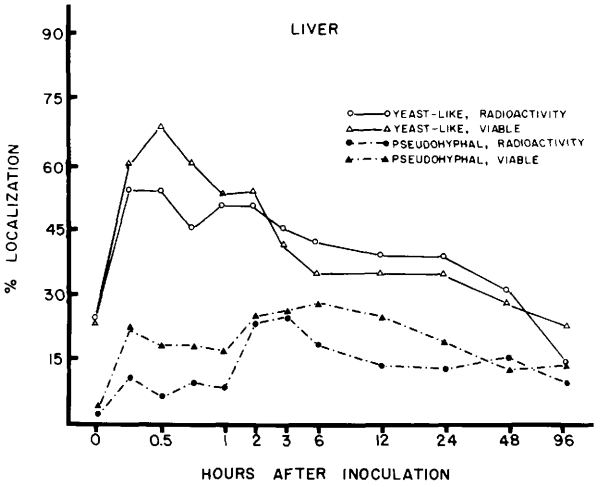

FIG. 2. Localization of radioactivity and viable units in the liver of AKR mice inoculated iv with 0.5 ml of a radioactive yeast-like (ATCC 10261) or pseudohyphal (ATCC 10259-R) suspensions of *Candida albicans*. Each point represents the mean for three animals. SE ranges from ±0.7 to ±13.5%.

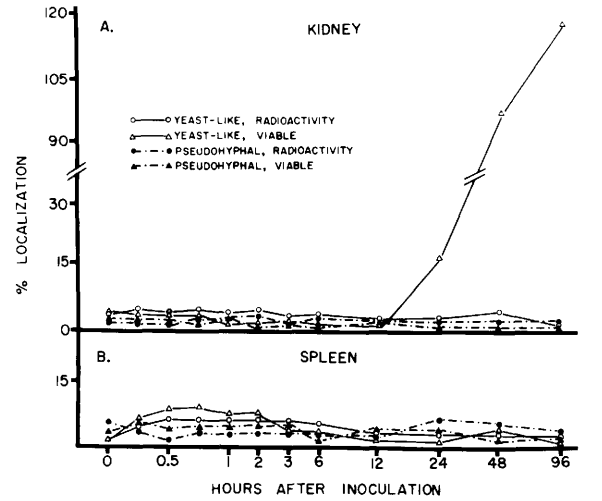

FIG. 3. Localization of radioactivity and viable units in (A) kidneys and (B) spleen of AKR mice inoculated iv with 0.5 ml of a radioactive yeast-like (ATCC 10261) or pseudohyphal (ATCC 10259-R) suspension of *Candida albicans*. Each point represents the mean for three animals. SE ranges from ±0.1 to ±30.7% (kidneys) and from ±0.1 to ±5.2% (spleen).

ble I is the ratio of radioactivity found in the lungs to that found in the liver. Although the lungs showed a greater trapping response than the liver for both morphological forms, the lung:liver ratio was initially much greater after challenge with the pseudohyphal organisms than after challenge with yeast-like cells. The number of labeled cells per milligram of spleen or kidney tissue (not shown) did not vary significantly between mice inoculated with yeast-like as compared with pseudohyphal preparations.

Discussion. The eventual outcome of infection may be related to the initial host management of intravascular microorganisms (5) but few studies have dealt with the role of major organs of the RES in host defense during systemic *Candida albicans* infections. Baine *et al.* showed that yeast cells of *C. albicans* injected into a peripheral vein of rabbits were cleared from the blood predominantly by the lungs but when the organisms were injected into a mesenteric vein they were largely cleared by the liver

TABLE I. ^{14}C IN LIVER AND LUNG TISSUE OF MICE AFTER CHALLENGE WITH LABELED *Candida albicans*.[a]

Hours after inoculation	Yeast-like (ATCC 10261)			Pseudohyphal (ATCC 10259-R)		
	cpm/mg of Tissue		Ratio	cpm/mg of Tissue		Ratio
	Lung	Liver	Lung:liver	Lung	Liver	Lung:liver
0	494	6	82	918	1	918
0.25	151	36	4.2	413	4	103
0.50	183	43	4.3	588	4	147
0.75	129	26	5.0	756	11	69
1	63	32	2.0	545	6	91
2	92	41	2.2	163	12	14
3	60	29	2.1	97	15	6.5
6	25	35	0.7	52	17	3.1
12	16	21	0.8	20	12	1.7
24	20	21	0.9	9	9	1.0
48	8	17	0.5	8	12	0.7
96	6	12	0.5	5	7	0.7

[a] Data are from a typical experiment in which mice were challenged iv with 0.5 ml of a yeast-like or pseudohyphal *Candida albicans* suspension, each containing approximately 2×10^5 cpm. Experiment was repeated three times with similar results.

(6). Rogers and Balish (7) found that *C. albicans* multiplied to a greater extent in the kidneys than in spleens, lungs, or livers of mice intravenously infected with the microorganism. Other investigators have examined *in vitro* phagocytosis of *C. albicans* by macrophages (8) or enhanced phagocytic activity of the RES following systemic *C. albicans* infections (9). However, the *in vivo* mechanisms by which the host deals with blood stream invasion by different morphological forms of this opportunistic pathogen have not been studied.

In our experiments a greater percentage of pseudohyphae localized initially in the lungs than did yeast-like cells, although both forms showed substantial trapping by lung tissue immediately after inoculation into a peripheral vein. Within 15 min after inoculation the number of yeast cells remaining in the lungs was drastically reduced and this reduction was accompanied by a simultaneous increase of the microorganisms in the liver.

Radioactive counts and viable units of both forms decreased at a much slower rate in the liver than in the lungs and substantial numbers of both forms remained in the liver at 96 hr. This finding suggests that the liver may be less effective than the lungs in killing both *Candida* strains. While the slow reduction of cell numbers in the liver may be due to killing and destruction of the microorganisms with concomitant release of radioactivity, it could alternatively be due to dissemination of the microorganisms, possibly within phagocytic cells, from sequestered areas in the liver. In fact, there is some *in vitro* evidence suggesting that invasiveness of *C. albicans* may be augmented through dissemination of the microorganism by the RES (10).

The kidney has been identified as the organ most severely affected during systemic candidiasis (11, 12) and our findings are consistent with this view. Relatively few cells of either form localized in the kidneys but the yeast cells that did so grew to produce an infection that resulted in death. On the other hand, when pseudohyphal cells were injected they appeared to be eliminated from the kidneys without subsequent growth and the animals survived. There are reports in the literature, however, which indicate that the yeast phase may initiate infections while pseudohyphae may be primarily associated with extension of a lesion (13).

Although there is evidence that the yeast-like form of *C. albicans* is more virulent than the pseudohyphal form, the underlying mechanisms involved in this phenomenon are not understood. It may be only fortuitous that the form with lesser virulence localizes in an organ of greater apparent killing capacity. Closely related but different strains of *Candida albicans* have been used in the present investigation and it is possible

that strain differences of the microorganism may partially account for some of the results reported here. However, preliminary experiments with the two morphological forms of strain 10261 (Evans and Mardon, unpublished data) indicate that pseudohyphae of this organism are of similar low virulence to strain 10259-R.

Summary. Localization of yeast-like and pseudohyphal cells of *Candida albicans* in various organs of AKR mice was examined following experimental fungemia. Relatively greater numbers of yeast-like than pseudohyphal organisms were cleared from the blood by the liver while more pseudohyphal than yeast-like cells localized in the lungs. Cells of both *C. albicans* forms disappeared more rapidly from the lungs than from the liver. Few cells of either form localized in the spleen or kidneys but the yeast cells found initially in the kidneys increased in numbers between 24 and 96 hr following injection. These data support the premise that disseminating systemic *C. albicans* infections are more likely to be initiated by the yeast-like form of the microorganism. The data also indicate that the lungs may play a vital role in innate host resistance against vascular dissemination of *C. albicans*.

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