PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 160, 6–10 (1979)

Disseminated Candidiasis in Leukopenic Dogs[1] (40377)

DANIEL V. EHRENSAFT, ROBERT B. EPSTEIN, SULEYMAN SARPEL, AND BURTON R. ANDERSEN

VA West Side Hospital 60612, Departments of Medicine and Microbiology, Abraham Lincoln School of Medicine, University of Illinois at the Medical Center, Chicago, Illinois 60680

Infection in the leukopenic host is a major cause of morbidity and mortality (1, 2). More effective antibacterial chemotherapy has led to a progressively increasing incidence of serious fungal infections (3–6). *Candida albicans* accounts for the majority of such infections in the compromised host and often presents difficult diagnostic and therapeutic problems (3–5). Disseminated candidiasis in granulocytopenic patients often presents with multisystem involvement (4).

Previous efforts to study *C. albicans* infection have involved the development of rodent systems in which the potentiating effects of such factors as antibiotics and corticosteroids were studied (7, 8). However, a large-animal model in which the effects of leukopenia on the development of disseminated candidiasis could be evaluated has not been previously reported. An experimental model of this type would provide a distinct advantage over one using small animals since granulocyte transfusions and other types of therapeutic and diagnostic manipulations would be easier to accomplish and more closely approximate the conditions in clinical infections. The present studies were carried out in canines to develop such a model.

Materials and methods. Candida preparation. A *Candida albicans* strain isolated from a patient's blood was grown overnight in trypticase soy broth at 37° in a shaking water bath. The yeast were centrifuged at 500*g* for 10 min and washed with saline three times. After each washing, the cell pellet was mechanically agitated for one minute to separate cell clumps. The cell concentration was adjusted to 10^7 yeast per ml (range 1.0–1.3 × 10^7) by direct hemocytometer count. Colony forming units of yeast per ml (range 0.9–1.5 × 10^7; Table I.) were determined by serially diluted agar pour plate counts.

Production of leukopenia. Mongrel dogs weighing between 15–20 kg and immunized against hepatitis and distemper were observed for two weeks prior to use. Leukopenia was produced by a single iv injection of cyclophosphamide (50 mg per kg body weight; courtesy of Mead Johnson Laboratories, Evansville, IN) as previously described (9). Dogs were fasted overnight prior to cyclophosphamide treatment and were given saline subcutaneously as needed for vomiting and dehydration.

Experimental design. Two groups of dogs were studied. Group one consisted of seven normal dogs while group two consisted of seven dogs rendered leukopenic by cyclophosphamide administered four to five days earlier. Both groups were challenged with 10^7 *Candida albicans* by intravenous injection. To prevent spontaneous bacteremia, five neutropenic animals were given gentamicin sulfate (2.5 mg/kg im; courtesy of Schering Corp., Kenilworth, NJ) and disodium carbenicillin (200 mg/kg sc; courtesy of Roerig Corp., N.Y., NY) twice daily. Antibiotics were started 12–24 hr before candida challenge and continued until death.

Followup procedures. Daily blood cultures were obtained. One tenth ml of heparinized blood was mixed with soft (56°) Mycobiotic agar (Difco Laboratories, Detroit, MI) and plated. Similar pour plates using blood agar base were obtained. This facilitated differentiation of *Candida albicans* from bacteria since the bacteria do not grow well on Mycobiotic agar, while yeast and bacteria both grow on blood agar base. Plates were incubated at 37°, and counted at 48 hr on a Quebec colony counter (American Optical Instrument Co., Buffalo, NY). Representative typical colonies were studied for germ tube formation to identify *Candida albicans*.

[1] This work was supported by Basic Institutional Support Funds, Veterans Administration West Side Hospital MRIS #0394-08 and 2456-07.

0037-9727/79/010006-05$01.00/0

TABLE I. QUANTITATIVE *Candida albicans* CLEARANCE.

Dog	Granulocytes per mm³ [a]	*C. albicans* Inoculum × 10^7	*C. albicans* colonies per ml of blood: Time (minutes)[b] 2	5	15	30	60	240
Control:								
6827	7700	1.2			NA[c]			<10
6422	10600	1.1	430	80	20	45	<10	10
6677	6300	1.5	1755	83	15	10	NG	NG
6568	13600	1.5	822	72	15	45	10	10
6722	11000	1.0	208	20	<10	NG	10	NG
6721	13800	1.0	670	55	NG[d]	10	NG	NG
5689	9200	0.9	510	30	10	10	10	NG
Neutropenic:								
5930	45	1.2			NA			NG
6724	70	1.4	2440	545	70	50	10	NG
6418	240	1.1	1052	275	30	15	<10	NG
6574	45	1.4	2362	700	130	60	<10	<10
6420	330	1.1	1200	125	10	<10	10	NG
6031	1374	1.0	598	135	40	20	<10	<10
6028	2170	1.0	960	195	55	10	<10	NG

[a] Granulocyte counts at the time of *C. albicans* challenge.
[b] Time following *C. albicans* injection.
[c] NA, Not available.
[d] NG: No growth.

Atypical colonies were gram stained.

Animals were autopsied as soon as possible after death. Control dogs were sacrificed at arbitrary intervals. In all dogs, samples of lung, liver, spleen, and kidney were obtained for culture and histologic examination. Bladder urine and heart blood were also collected. Hematoxylin and eosin and silver methenamine stains of tissue sections were performed.

Weighed samples of tissues were homogenized in 5 ml sterile saline. Pour plate cultures of blood, urine, and tissue homogenate dilutions were obtained. Organisms were counted and identified by the same methods used for the daily blood cultures.

Results. Table I shows the blood clearance of *C. albicans* following intravenous challenge in normal control and leukopenic dogs. The initial disappearance is logarithmic in both groups with significantly higher colony counts in leukopenic dogs during the first 15 minutes. By 60 min, levels of candida were similar. Blood cultures were negative in both groups at 24 hr.

All animals with granulocyte counts less than 500 mm³ died within 72 hr (Table II). Two neutropenic dogs with initial granulocyte counts above 1000mm³ lived four and 6 days respectively. Control animals remained clinically well until sacrifice.

At autopsy leukopenic dogs showed a consistent picture of disseminated candidiasis. Grossly 1–3 mm circumscribed white lesions were observed throughout the heart, liver, spleen and kidney. Control animals demonstrated no gross pathology at sacrifice 2–10 days post candida challenge.

Microscopic evaluation of multiple tissue sections confirmed the gross findings of extensive candida organ involvement. Lesions appeared as circumscribed areas of pseudohyphae and yeast (Fig. 1). Leukocyte infiltration was notably absent except for occasional collections of mononuclear elements around some lesions. Sites of candida infiltration were termed pseudoabscesses because of the lack of granulocytes in involved areas. Tissues of control dogs showed no microscopic evidence of infection.

Quantitative autopsy cultures for blood, urine, lung, liver, spleen and kidney are shown in Table II. Blood cultures were positive for 10 or less organisms per ml in five neutropenic dogs and were negative in two despite the extensive tissue involvement in all of these animals. The urine showed large numbers of candida (10^4–10^5 per ml) in four dogs tested.

Most of the tissue cultures in control dogs were sterile. Of interest were the occasional

TABLE II. QUANTITATIVE CULTURES AT AUTOPSY.

Dog	Survival post challenge[b] Days	*C. albicans* colonies/ml		*C. albicans* colonies/g wet tissue			
		Blood	Urine	Lung	Liver	Spleen	Kidney
Control:							
6827	2	NG[c]	NG	NG	10^2	10^2	NG
6422	3	NG	NG	NG	10^2	10^2	10^2
6677	7	NG	NG	NG	NG	NG	10^3
6568	7	NG	NG	<10	<10	NG	NG
6722	10	NG	NG	NG	NG	NG	NG
6721	10	NG	NG	NG	NG	<10	NG
5689	10	NG	NG	NG	NG	NG	NG
Neutropenic:							
5930	1	<10	10^5	10^6	10^5	10^6	10^7
6724	1[a]	<10	10^5	10^4	10^4	10^5	10^4
6418	2	NG	NA[d]	10^4	10^4	10^5	10^5
6574	2	10	10^4	10^5	10^5	10^5	10^6
6420	2[a]	10	NA	10^4	10^4	10^4	10^7
6031	4[a]	NG	>10^5	10^4	10^3	10^5	10^6
6028	6[a]	<10	NA	10^3	10^5	10^5	10^7

[a] Animal found dead in morning.
[b] Control animals sacrificed at time indicated.
[c] NG: No growth.
[d] NA: Not available.

positive cultures isolated from the organs of control animals, particularly those sacrificed within 72 hr following challenge. Quantitative cultures showed few organisms compared to neutropenic dogs and were negative in those dogs sacrificed at later times. Extensive involvement of the lung, liver, spleen and kidney were present in the neutropenic animals. Among the cultured tissue, the kidney was the site of major involvement.

Two neutropenic dogs not receiving antibiotics (6418 and 6420) developed mixed bacterial and candida infections. Quantitative cultures for candida were similar to antibiotic treated dogs that failed to develop intercurrent bacterial infections.

Thus normal dogs were able to control the standard challenge of 10^7 organisms, while neutropenic animals developed a consistent picture of disseminated candidiasis.

Discussion. The present studies establish a canine model for consistently producing disseminated candidiasis. At the challenge dose of *C. albicans* used, widespread tissue infection depended on the production of severe leukopenia and did not occur in the hematologically normal host. Modest reductions in initial blood clearance rates in leukopenic dogs make definitive conclusions regarding the pathophysiologic participation of granulocytes and/or the macrophage system difficult to separate at present. However, it has been demonstrated in the cyclophosphamide treated dog that granulocytopenia is crucial in predisposing to spontaneous or induced infections, and that granulocyte transfusions alone are beneficial (9, 10). Studies of replacement therapy (e.g., granulocyte transfusions) will be of interest in the present model. Administration of antibiotics was useful in the system to prevent intercurrent bacterial infection so that the candida infection could be studied independently.

Death occurred in one to six days following the intravenous challenge of 10^7 organisms in leukopenic dogs. As seen in a leukopenic murine model (11), survival was associated with the degree of neutropenia at *C. albicans* challenge. In contrast to previously reported bacterial sepsis models in the dog (9, 12) a prominent secondary wave of fungemia failed to develop following initial blood clearance. Only occasional organisms were isolated from the blood at the time of death. A similar absence of fungemia is common in disseminated candidiasis in man (3, 4, 13). Concurrent bacterial infection and systemic candidiasis may be seen in man. The present studies show that uninhibited disseminated candidiasis occurred in those animals with concurrent bacterial sepsis.

Tissue involvement by candida pseudoab-

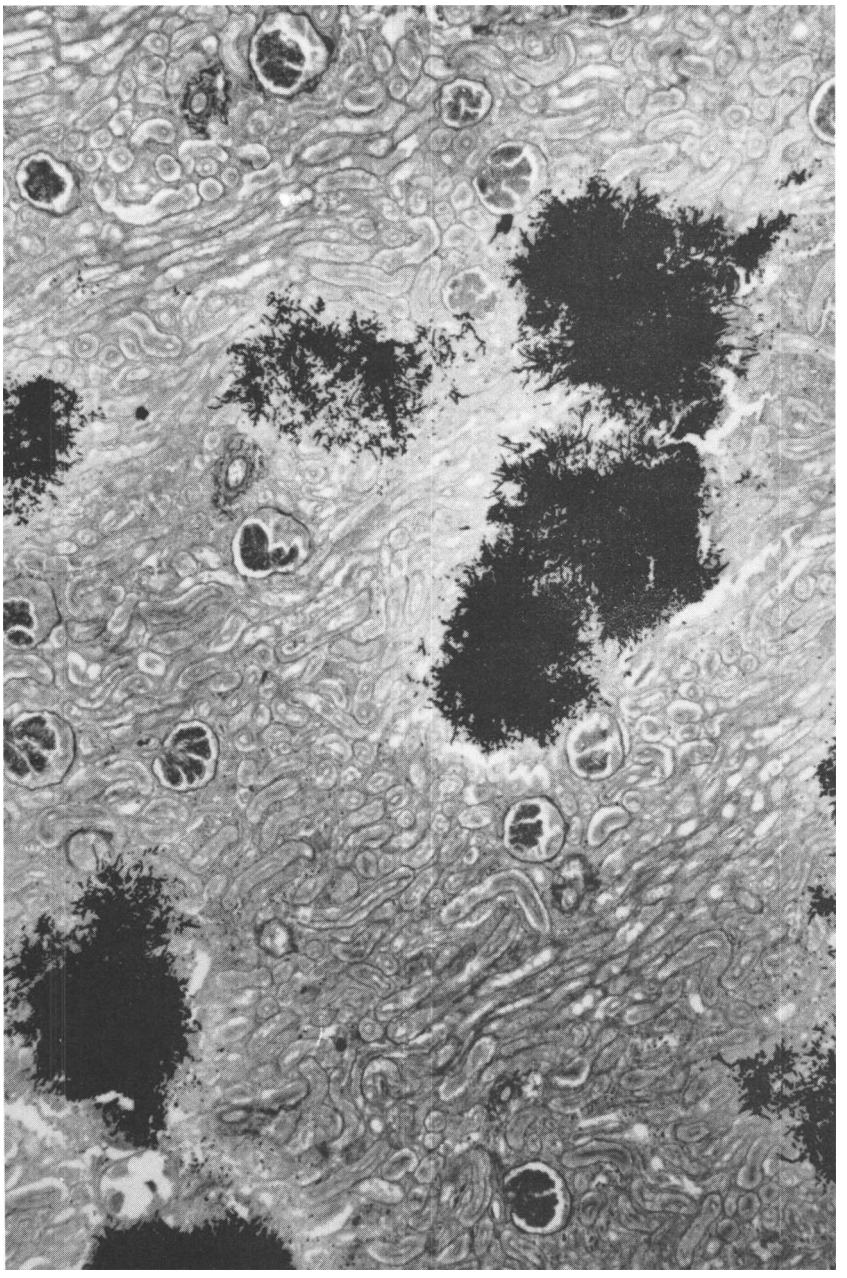

FIG. 1. Kidney of dog 5930 demonstrating several circumscribed areas consisting predominantly of pseudohyphae with occasional yeast (pseudoabscess). Note absence of inflammatory cell infiltrate. Silver methanamine (×125)

scesses was widespread and was particularly extensive in the kidneys. This has been found in small-animal models and man (5, 7, 13, 14). Louria et al (7) suggest that the predilection for candida infection by the kidney is related to hyphal proliferation inside renal tubules, where there exists a sanctuary from host polymorphonuclear response. Since in the present study the granulocyte pool is severely diminished, other mechanisms may be responsible.

Although candiduria may only reflect colonization or localized lower urinary tract infection, the present studies confirm the frequent occurrence of candiduria as an indication of disseminated disease (3–5).

The granulocyte response to candida tissue invasion is well established in animals and man (7, 8, 13, 14). The granulocytopenic host would, therefore, be expected to have difficulty contending with such infections. This assumption is supported by the present studies as well as human data (3, 4).

The reproducibility of the clinical course, microbiology and pathology of the leukopenic-dog model provides a large-animal system for the study of disseminated candidiasis.

Summary. Fourteen dogs were intravenously challenged with 10^7 *Candida albicans.* Seven of these animals were rendered leukopenic with cyclophosphamide. Both groups cleared organisms from the circulation. Normal dogs remained well and showed no gross or microscopic evidence of candidiasis at autopsy. In contrast, leukopenic animals died 1–6 days after receiving *C. albicans* and demonstrated a consistent picture of disseminated candidiasis. Features of this model similar to human infection include regular candiduria but only occasional candidemia despite severe tissue involvement. The reproducibility of this model system provides a basis for *in vivo* investigation of systemic fungal disease in the compromised host.

The authors thank Bernard Tegtmeier, Barbara Cunningham, Yvonne Taylor, and Ruth Hostler for technical assistance.

1. Bodey, G. P., Buckley, M., Sathe, Y. S., and Freireich, E. J., Ann. Intern. Med. **64,** 328 (1966).
2. Inagaki, J., Rodriquez, V., and Bodey, G. P., Cancer. **33,** 568 (1974).
3. Bodey, G. P., J. Chron. Dis. **19,** 667 (1966).
4. Hart, P. D., Russell, E., Jr., and Remington, J. S., J. Infec. Dis. **120,** 169 (1969).
5. Myerowitz, R. L., Pazin, and G. J., Allen, C. M., Amer. J. Clin. Pathol. **68,** 29 (1977).
6. Winter, W., and Foley, G., Pediatrics **18,** 595 (1956).
7. Louria, D. B., Fallon, N., and Browne, H. G., J. Clin. Invest. **39,** 1435 (1960).
8. Seelig, M. S., Amer. J. Med. **40,** 877, (1966).
9. Epstein, R. B., Waxman, F. J., Bennett, B. T., and Andersen, B. R., Transfusion. **14,** 51 (1974).
10. Debelak, K., Epstein, and R., Andersen, B., Blood **43,** 757 (1974).
11. Mukherji, A. K., and Basu Mallick, K. C., Indian J. Med. Res. **60,** 1584 (1972).
12. Epstein, R. B., Clift, R. A., and Thomas, E. D.: Blood **34,** 782 (1969).
13. Louria, D. B., Stiff, D. P., and Bennett, B., Medicine **41,** 307 (1962).
14. Hurley, D. L., and Fauci, A. S., J. Infec. Dis. **131,** 516 (1975).

Received July 10, 1978. P.S.E.B.M. 1979, Vol. 160.