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Experimental Murine Candidiasis: Pathological and Immune Responses to Cutaneous Inoculation with *Candida albicans*

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Cutaneous infection of mice with *Candida albicans* elicited a predominantly acute inflammatory response, stimulated the production of precipitating antibodies, and conferred protection against subsequent intravenous challenge with the same organism. The acute inflammatory skin reaction seen after cutaneous infection suggested a predominantly humoral response to *Candida*. Animals infected cutaneously a second time with viable *C. albicans* developed larger skin lesions than animals infected only once, and the twice-infected animals were more resistant to an intravenous challenge as well. The cutaneous inoculation of mice with heat-killed *C. albicans* was less effective in stimulating antibody production, in eliciting the inflammatory response, and in inducing a protective response demonstrable by intravenous challenge with viable *Candida*. This model of experimental candidiasis represents a reproducible means of studying a protective immune response to the organism.

Candida albicans is a ubiquitous fungus, regularly colonizing normal individuals without initiating overt disease (3). The mechanisms by which normal people resist disease are poorly understood, but since individuals with abnormalities affecting their cellular immune systems are particularly susceptible to candidiasis (2, 16-18, 33), usually in a chronic mucocutaneous form, it has been thought for some time that the primary defense against *Candida* involves that arm of the immune system. To the contrary, however, there have been reports of increased resistance to systemic candidiasis in animals devoid of an intact cellular immune system (7, 30), and successful treatment with immune serum has been reported in man (15) and experimental animals (1).

Our interest has been in the area of acquired rather than innate resistance, and one of the drawbacks to the study of acquired resistance in candidiasis has been the lack of a suitable animal model, i.e., one in which protective responses have been induced by infection and one in which the immune responses have been defined. It was our initial intention to devise a model of self-limited infection that would be reproducible and quantifiable and in which resistance to reinfection could be assessed without the use of a lethal challenge infection. We patterned preliminary studies after those of Preston and Dumonde (24) wherein increased resistance to reinfection could be detected in mice simply by comparing cutaneous lesions resulting from the inoculation of *Leishmania* both into previously uninfected animals and into animals that had recovered from a prior infection with the same organism. The latter animals developed lesions that were smaller and resolved more quickly than their previously uninfected counterparts. As our investigation progressed, however, it was not possible to use such differences in lesion size alone to assess protection, although immune responses were induced by cutaneous infection and protection could be demonstrated by intravenous challenge.

MATERIALS AND METHODS

Cultural methods. *C. albicans* B311, serotype A, obtained from H. Hasenclever, was maintained by monthly transfer on glucose-peptone agar slants and stored at 3 to 5°C. The culture was checked periodically for biochemical and morphological conformity to established criteria (11, 28).

Viable yeast cells for animal inoculation or antigen preparation were obtained by transferring growth from a glucose-peptone agar slant that had been incubated at 37°C for 24 h to 100 ml of soy dialysate broth (27). The broth culture was then incubated at 37°C on a gyratory shaker (New Brunswick Scientific Co., New Brunswick, N.J.) at 160 rpm for 16 to 17 h. The cells were harvested by centrifugation and washed three times with nonpyrogenic physiological sodium chloride (NPS) (Cutter Laboratories, Berkeley, Calif.). More than 95% of the cells in such suspensions were blastospores. The cells were then quantitated in a hemacytometer, and the counts were verified by diluting and plating the same suspensions with which animals were inoculated. Killed cells for animal inocula-

tion were prepared by heating 20 ml of a suspension containing 1×10^8 to 2×10^8 washed cells per ml at 60°C for 30 min. For use, the suspension was diluted with NPS to a final concentration of 1.0×10^7 blastospores per ml.

Kidneys were removed aseptically, and both kidneys from one animal were homogenized in a Potter tissue grinder. Appropriate dilutions were plated in triplicate on glucose-peptone agar containing 300 μg of chloromycetin per ml.

Cell fractionation procedures. Blastospores of *C. albicans* that had been grown and harvested as described above were disrupted in a Braun homogenizer (model MSK; Bronwill Scientific Co., Rochester, N.Y.) using 0.25- to 0.30-mm-diameter glass beads, and the resulting mixture was separated by sequential centrifugations as described by Domer (10). The disrupted cells were decanted from the glass beads and centrifuged at $450 \times g$ for 30 min. The supernatant fluid was then centrifuged at $20,000 \times g$, decanted from the pellet, and centrifuged again at $144,000 \times g$ for 120 min. The pellet resulting from the low-speed centrifugation contained mainly cell walls, whereas that pellet resulting from the intermediate-speed centrifugation contained membranes, mitochondria, mitochondrial ribosomes, and smaller cell wall fragments. The latter pellet is referred to hereafter as MM. The high-speed centrifugation resulted in a pellet containing ribosomal material (RR) and a supernatant containing the soluble cytoplasmic substances (SCS). MM and SCS fractions were lyophilized and stored over a desiccant. In preliminary studies, the MM was shown to be the most reactive in footpad assays, while the RR and SCS fractions did not elicit delayed reactions. The SCS fraction, however, was reactive in precipitin tests. Since the MM fraction was insoluble, an effort was made to obtain a soluble preparation from it that would detect delayed responses. Accordingly, the MM fraction was incubated at 50°C for 60 min in phosphate-buffered saline, pH 7.4, and 100 mg of MM per 10 ml of saline. The mixture was then centrifuged at $20,000 \times g$ for 30 min, and the supernatant was dialyzed against distilled water at 4°C for 48 h. It was concentrated by dialysis against 30% (wt/vol) polyethylene glycol 20,000 (J. T. Baker, Co., Phillipsburg, N.J.) or by ultrafiltration (Amicon PM-10 filter, Amicon Corp., Lexington, Mass.). Protein content was estimated by the method of Lowry et al. (19) and carbohydrate by the anthrone method (31). The extract, referred to as the heat-extracted or HEX fraction, was stored at −20°C at a concentration of 1 mg of protein per ml. It contained approximately 0.5 mg of carbohydrate per ml. The SCS fraction contained approximately 75% protein and 13% carbohydrate.

Cutaneous and intravenous inoculation. CBA/J mice (Jackson Laboratories, Bar Harbor, Me.) were used throughout these studies. They were numbered by ear punch and randomized for each experiment. Each animal was bled before infection. Twenty-four hours before inoculation, an area approximately 2 by 3 cm in size was clipped on the flanks of those mice to be inoculated cutaneously. Group size for all experiments was 15 to 20 mice. For cutaneous inoculation, the appropriate number of *Candida* cells was injected intradermally into the shaved flank, and the height and diameter of the resulting lesions were measured daily with a dial-type direct-reading vernier caliper (model 579-10; Brown and Sharpe Manufacturing Co., North Kingston, R.I.) Using these two measurements, the volume of each lesion was calculated by the formula $V = 4/3\,\pi r^2 t$, where r is the average radius, t is the height, and V is the volume of an oblately ellipsoid object. For intravenous infections, the desired number of viable cells was suspended in 0.5 ml of NPS and injected into a lateral caudal vein.

Histopathology. Mice were sacrificed, and either the dermal lesion with surrounding tissue or the foot that had been inoculated with antigen was removed and fixed in buffered 10% formalin. Tissues were embedded in paraffin, and sections were cut and stained with hematoxylin and eosin or periodic acid-Schiff stain (13). Stained sections of dermal lesions were randomly coded and evaluated without knowledge of the experimental design for the presence of chronic or acute inflammation, infiltration of blast cells or plasma cells, the presence of intact fungal organisms, fibrosis, and the condition of the overlying epidermal surface.

Serological methods. Pre- and postimmune sera were tested for antibodies to the SCS fraction of *Candida* by counterimmunoelectrophoresis (CIE). Selected sera were also tested with two commercial preparations: Hollister-Stier *Candida* (*Monilia*) *albicans* allergenic extract 1:10 (Hollister-Stier Laboratories, Spokane, Wash.) and *C. albicans* allergenic extract (Greer Laboratories, Lenoir, N.C.). The CIE technique used was similar to that of Remington et al. (26) and Gordon et al. (12). Positive control serum from a hyperimmune guinea pig infected and boosted with viable *C. albicans* B311 emulsified in complete Freund adjuvant was included with each electrophoretic run. SCS was used as antigen at a concentration of 5 mg (dry weight) per ml of barbitone-tris(hydroxymethyl)aminomethane buffer. Slides were washed in 2% sodium chloride in 0.05 M phosphate buffer, pH 7.2, overnight and in 0.05 M phosphate buffer for 3 h, and then dried and stained with amido black (5).

Qualitative agglutinations (32, 35) were performed on sera in parallel with the CIE, using 2.0×10^7 *C. albicans* yeast cells per ml as antigen.

Footpad testing. The footpad swelling test was performed in a manner similar to that described by Cooper (4) and Crowle (6). The HEX antigen was adjusted to 20 μg of protein per test dose, and that dose was administered in 20 μl. Foot thickness was measured with a dial-type caliper (Schnelltäster; H. Kröplein GmbH, Schluchtern, Hesse, West Germany) before the injection of antigen and at 4, 7, 24, and 48 h postinjection. The net increase in foot thickness at each time interval was determined by subtraction, and the mean net increase in footpad thickness of the control animals was subtracted from the mean experimental animal values.

RESULTS

Gross pathology in mice after intracutaneous inoculation with *C. albicans*. Preliminary experiments were designed to determine the number of viable blastospores for intracu-

taneous inoculation that would result in a reproducible, quantifiable lesion of sufficient duration to permit optimal stimulation of immune responses of experimental animals. Groups of 15 mice were inoculated cutaneously with either 1.0×10^5, 5.0×10^5, 1.0×10^6, or 1.0×10^7 viable blastospores. The lesions that developed after inoculation with 1.0×10^5 blastospores were small and measurement was difficult, whereas the lesions produced after inoculation of 1.0×10^7 blastospores were initially quite large but ulcerated within a few days. Ulceration promoted healing and resulted in wide variations in lesion size within the group. Inoculation of 5.0×10^5 or 1.0×10^6 blastospores resulted in lesions of reproducible and quantifiable size that required 2 or 3 weeks to resolve. Mean lesion volumes in response to the inoculation of each of these two doses into previously uninfected mice are presented as the lower curves in each frame in Fig. 1. A photograph of a typical cutaneous lesion 3 days after inoculation of *Candida* into a previously uninfected mouse is presented in Fig. 2. In preliminary experiments, attempts were made to culture *Candida* from draining lymph nodes and various organs of mice infected with 1.0×10^6 blastospores. None of the cultures were positive. Since 5.0×10^5 and 1.0×10^6 blastospores resulted in lesions that met the criteria defined above, both doses were used in subsequent experiments to determine the effect of one cutaneous inoculation on a second cutaneous inoculation, or the effect of one or two cutaneous inoculations on subsequent intrave-

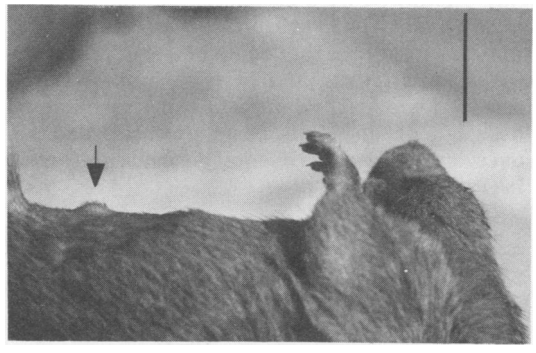

FIG. 2. *Representative lesion (arrow) resulting from the cutaneous inoculation of 5×10^5 viable C. albicans into a previously uninfected mouse 3 days before photography. Bar = 1 cm.*

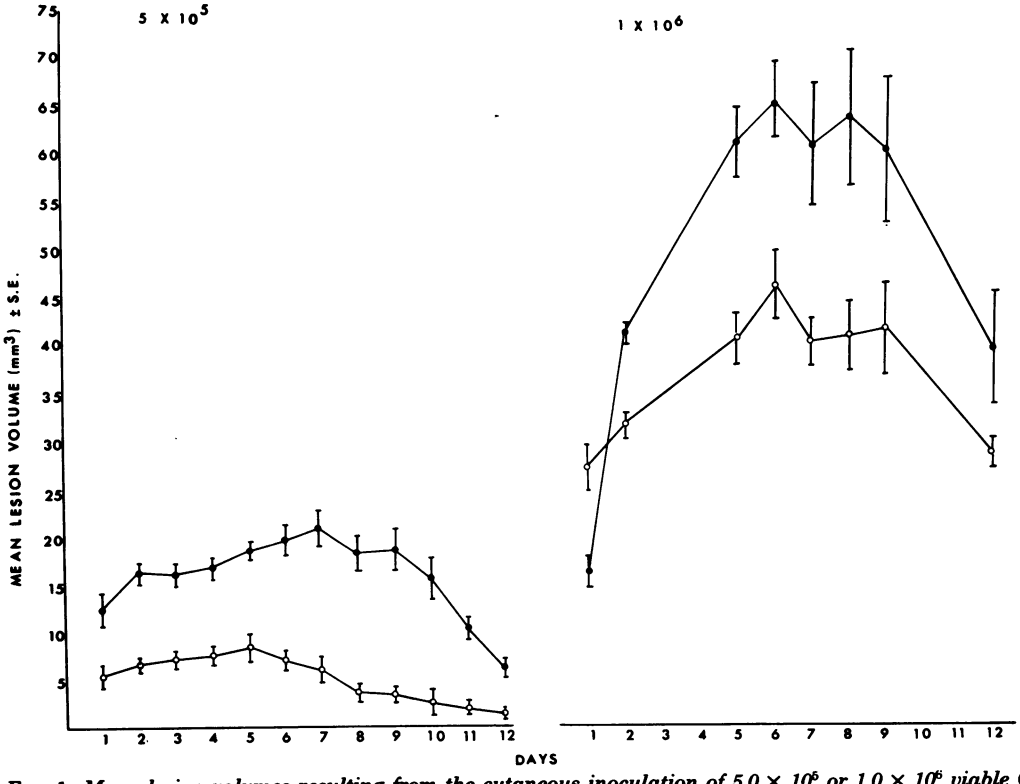

FIG. 1. *Mean lesion volumes resulting from the cutaneous inoculation of 5.0×10^5 or 1.0×10^6 viable C. albicans blastospores into previously uninfected* (○) *or previously infected* (●) *mice. n = 15.*

nous challenge. Contrary to what had been anticipated, i.e., smaller cutaneous lesions in animals with prior exposure to *Candida*, animals that had been infected cutaneously once developed larger lesions when challenged by the same route a second time (Fig. 1).

As the research evolved, it became apparent that the sequence of cutaneous inoculations described above, i.e., one cutaneous inoculation on day 0 followed by a second cutaneous inoculation on day 14, was suitable for stimulating immune responses that could be detected in vivo by footpad testing and in vitro by serological assays. Therefore, subsequent experiments were designed around that basic protocol, and an overview of the experimental design is presented in Table 1.

Since the difference in size between the lesions that resulted from a first infection and those that developed upon reinfection could have been the result of a delayed-type hypersensitivity response superimposed on a nonspecific inflammatory response, we decided to inoculate groups of mice with various sequences of live and killed blastospores, using the basic experimental design presented in Table 1. Five groups of 20 mice each were inoculated in the following sequences: (A) live on day 0, live on day 14, (B) killed on day 0, live on day 14, (C) nothing on day 0, live on day 14, (D) live on day 0, killed on day 14, (E) killed on day 0, killed on day 14. Cutaneous responses observed after the second inoculations are presented in Fig. 3.

Mice inoculated only once with 5.0×10^5 viable

TABLE 1. *Summary of basic experimental design*

Day	Group			
	I	II	III	IV
−1	Bled	Bled	Bled	Bled
0 (first inoculation)	Inoculated cutaneously[a]	Inoculated cutaneously		
13	Bled			
14 (second inoculation)	Inoculated cutaneously		Inoculated cutaneously	
27	Bled	Bled	Bled	
28	All animals were either footpad tested for evidence of delayed hypersensitivity or challenged intravenously with viable blastospores to assess protection.			

[a] Cutaneous inoculations consisted of either 5×10^5 or 1×10^6 viable or heat-killed blastospores.

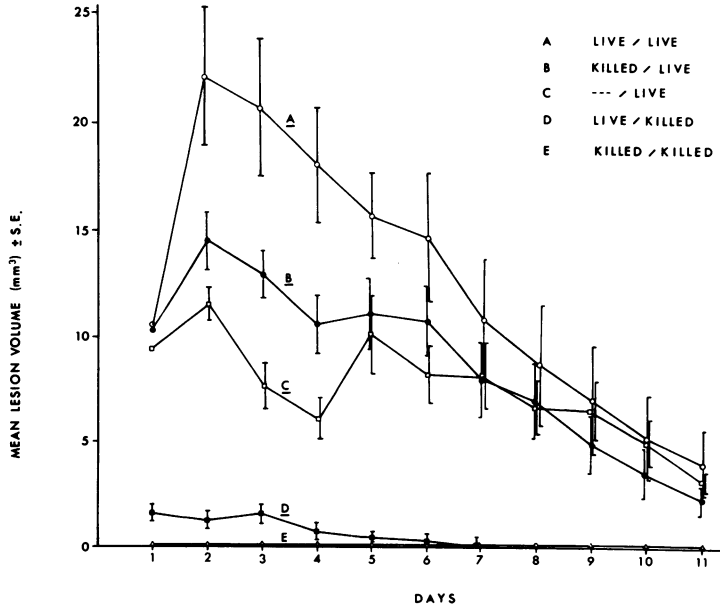

FIG. 3. *Mean lesion volumes resulting from the cutaneous inoculation of viable or heat-killed blastospores of C. albicans into mice inoculated 2 weeks previously with viable or heat-killed blastospores (A,B,D,E). Previously uninoculated mice were inoculated once with viable C. albicans blastospores (C). n = 20.*

blastospores (group C) developed lesions similar to those described above and presented in Fig. 1. On the other hand, mice inoculated only once with killed blastospores (not shown) never developed a measurable lesion. As seen in earlier experiments, when both inoculations were live (group A), the second lesion was considerably larger than that produced in previously uninfected animals by the same dose. Animals inoculated twice with killed blastospores (group E) did not develop measurable lesions at any time. Mice inoculated first with killed blastospores and then with viable *Candida* (group B) developed lesions after the viable challenge that were similar in volume to those of mice receiving a viable inoculation for the first time. If mice were inoculated first with viable and then with killed blastospores (group D), small but measurable dermal lesions developed after inoculation of the killed suspension. Although not shown, similar data were obtained from animals given the same combinations of dead and live inoculations with 1.0×10^6 blastospores. From these data, it can be concluded that heat-killed inocula injected cutaneously stimulate relatively poor immune responses, and that the enlarged lesions that developed in response to a second live inoculation were probably not due solely to a delayed hypersensitivity reaction resulting from the introduction of antigenic material, in the form of killed cells, superimposed on nonspecific inflammation.

Histopathology of cutaneous lesions. Cutaneous lesions were removed from mice inoculated once or twice with live and/or killed blastospores. In all animals inoculated with viable blastospores, an abscess formed in the deep dermal tissue and was surrounded by a mixed acute and chronic inflammatory reaction. Most lesions had concentrations of foamy histiocytes, but they were never aggregated into a granuloma. Giant cells were not present. The only discernible differences between animals inoculated once with viable *Candida* and those inoculated twice was in the intensity of the cellular infiltrate and in the numbers of blastospores or mycelial elements observed within the abscesses. There seemed to be greater numbers of fungal elements in lesions from mice previously exposed to *Candida* than in mice exposed for the first time. Attempts to demonstrate the latter observation by culture were inconclusive; i.e., in one of two experiments there were larger numbers of colony-forming units when the lesions were cultured 1, 3, and 6 days after inoculation. Nine days after inoculation, however, in both experiments, the number of viable colony-forming units in lesions from animals previously infected dropped well below those of animals being challenged for the first time.

Animals inoculated with live blastospores could readily be distinguished from those inoculated with killed organisms, since intact fungal elements were observed only in those animals given live inocula. The histological differences between reactions to live and dead *Candida* were, in general, more striking than the differences between first and second inoculations with viable *Candida*: an abscess cavity frequently (79%) developed in response to a second inoculation with viable *Candida*, whereas it did not develop after an inoculation with dead *Candida*, even in animals that had been previously infected.

Intravenous challenge of uninfected and previously infected mice. Since it was difficult to interpret resistance to reinfection by cutaneous challenge (i.e., smaller lesions of shorter duration did not occur upon reinfection), we decided to explore the possibility that cutaneous infections induced protective responses that could be measured by intravenous challenge. First, however, the virulence of the strain of *Candida* being used was determined by challenging previously uninfected mice with doses of *Candida* ranging from 1.0×10^4 to 1.0×10^6. All animals receiving 1.0×10^6 or 5.0×10^5 blastospores died within 5 days of inoculation. Since 50% of the animals receiving 1.0×10^5, 5.0×10^4, and 1.0×10^4 died within 9, 18, and 25 days of challenge, respectively, those doses were subsequently used to determine whether protective responses had been induced in mice by two cutaneous inoculations with viable blastospores. Further, in the experiment in which 1.0×10^4 blastospores was the intravenous challenge dose, mice that had been infected cutaneously only once, 2 or 4 weeks before intravenous challenge, were also included in the protocol (Fig. 4).

When challenged intravenously with 1.0×10^5 blastospores, all mice, regardless of prior exposure to *Candida*, died within 10 days of challenge (Fig. 4A). On the other hand, increased survival rates were observed in mice infected cutaneously twice if the intraveous challenge dose was lowered to 5.0×10^4 or 1.0×10^4 blastospores (Fig. 4B, C). Animals infected cutaneously once 2 weeks before intravenous challenge were protected as well, but animals infected cutaneously once 4 weeks before intravenous challenge were not protected. The experiments with challenge doses of 5.0×10^4 and 1.0×10^4 were repeated with similar results, except that the degree of protection induced by a single cutaneous inoculation 2 weeks before intravenous challenge was variable.

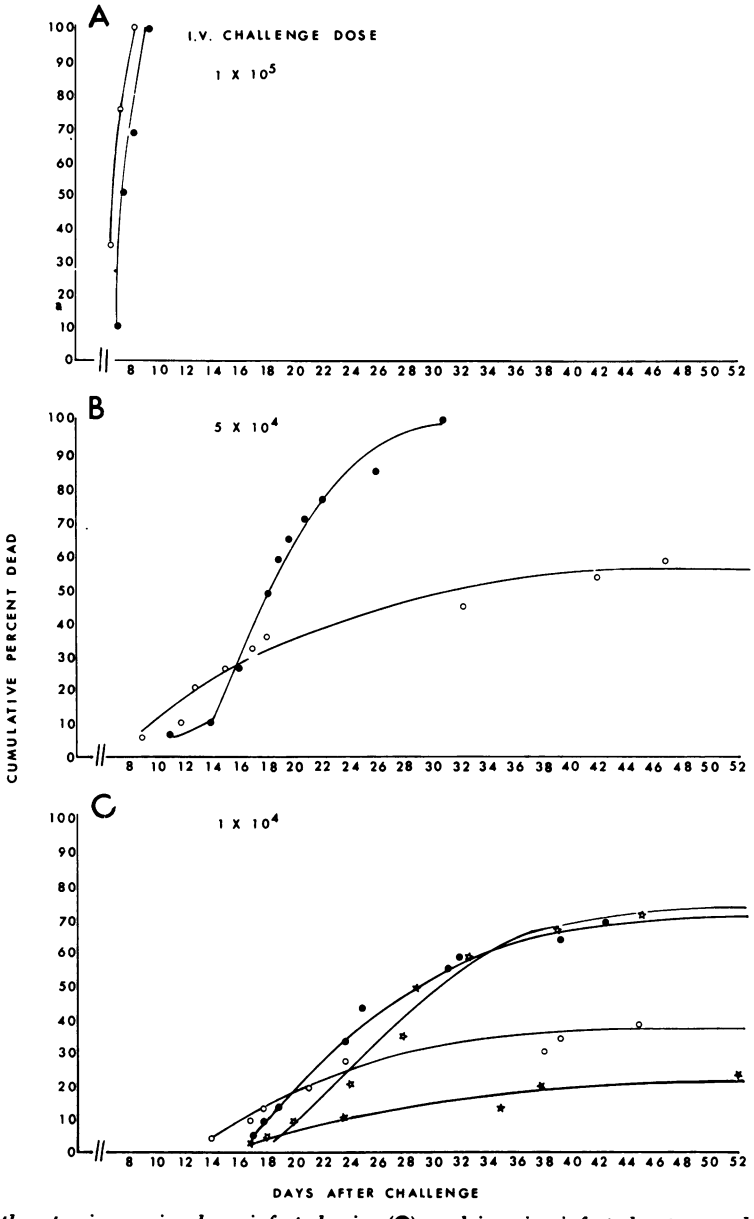

FIG. 4. *Death rates in previously uninfected mice* (●) *and in mice infected cutaneously with 1.0 × 10⁶ viable C. albicans blastospores either twice before intravenous challenge* (○), *once 4 weeks before intravenous challenge* (☆), *or once 2 weeks before intravenous challenge* (★). *n = 20.*

All animals living 52 days after intravenous challenge were sacrificed, and their kidneys were cultured. Only 10% of those animals infected cutaneously twice and then challenged intravenously with 5.0×10^4 blastospores were culturally negative, despite the fact that 50% of the original number were still alive 52 days after challenge. To the contrary, 40 to 60% of the kidneys of mice infected cutaneously twice, or once 2 weeks before intravenous challenge, were culturally negative if the intravenous challenge dose was 1.0×10^4. All of the surviving previously uninfected mice challenged intravenously with 1.0×10^4 blastospores, as well as all surviving animals infected cutaneously once 4 weeks before intravenous challenge, had *Candida* in their kidneys.

Since the possibility existed that the protec-

tion observed in animals infected twice cutaneously could have resulted from the introduction of immunogenic material rather than from an active infection, animals that had been given live and dead inocula in various combinations were challenged intravenously (Fig. 5). Approximately equal levels of protection were observed in all animals given two inoculations providing at least one of those inoculations was live. Killed inocula alone induced little protection, as did a single viable inoculum administered 2 weeks before intravenous challenge. It would appear, therefore, that while killed cells can either stimulate or boost a protective response, live cells are required to stimulate a substantial response.

Immune responses in cutaneously infected mice. Sera obtained from mice before and after inoculation with live or dead *Candida* were tested for precipitating and agglutinating antibodies. The data obtained from the CIE analyses are presented in Table 2. Most of the positive reactions consisted of one or two precipitin lines. The SCS antigen was the most sensitive of those tried. HEX and the commercial antigens were less reactive. To emphasize the decreased sensitivity of the commercial preparation, data obtained when the same sera were tested in parallel with SCS and one of them, the Greer antigen, are presented in Table 2. SCS detected 2 to 10 times more positive reactions. None of the animals had detectable precipitins before inoculation, and only 30% had developed precipitins 13 days after one cutaneous inoculation with 1.0×10^6 viable blastospores when tested with SCS. Ten percent or less developed precipitins under the same conditions when inoculated cutaneously with 5×10^5 viable blastospores. On the other hand, 50 to 90% of all mice had developed precipitins when reinfected on the flank with a viable inoculum. Only 10% of the mice inoculated twice with killed blastospores had developed antibodies 2 weeks after the second inoculation. In all instances where one or the other of the inocula

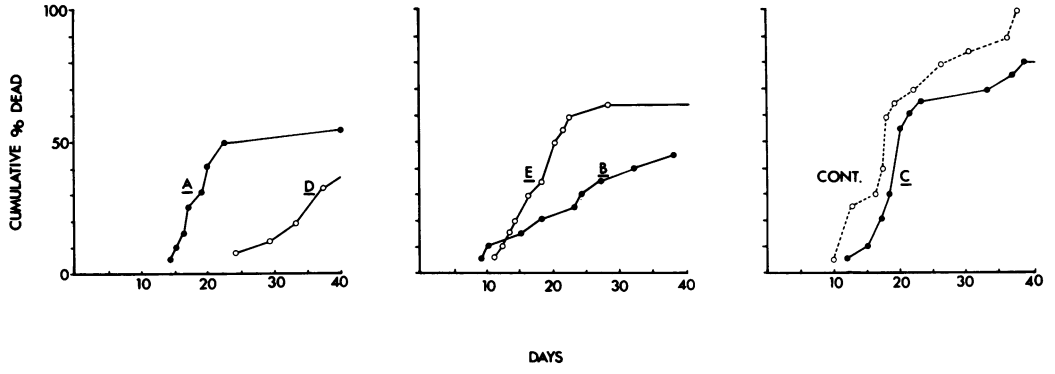

FIG. 5. *Death rates in previously uninoculated mice (CONT) and in mice having been inoculated cutaneously once or twice with viable or heat-killed blastospores of C. albicans. See Fig. 3 or 6 for explanation of A-E.*

TABLE 2. *Summary of CIE results with sera from mice inoculated once or twice with viable and/or heat-killed blastospores of C. albicans*

Dose	Inoculum (first/second)	Antigen[a]	Precipitins detected		
			Preinoculation	13 Days after first inoculation	13 Days after second inoculation
1.0×10^6	Live/live	SCS	0/115[b,c] (0)[d]	34/113 (30.0)	36/39 (92.3)
		Greer	0/115 (0)	3/112 (2.7)	19/38 (50.0)
5.0×10^5	Live/live	SCS	0/155[c] (0)	9/155 (5.8)	59/110 (54.0)
	Live/killed	SCS	0/18 (0)	ND	1/17 (5.9)
	Killed/live	SCS	0/20 (0)	0/20 (0)	0/20 (0)
	Killed/killed	SCS	0/20 (0)	ND	2/20 (10.0)
	—/live[e]	SCS	0/20 (0)	3/20 (15.0)	

[a] SCS, Soluble cytoplasmic substances; Greer, *C. albicans* allergenic extract from Greer Laboratories. Those sera were tested in parallel with each antigen.

[b] Number of positive sera/number of sera tested.

[c] Several experiments combined.

[d] Numbers in parentheses indicate percent positive.

[e] Control mice for the second inoculation with live organisms.

was killed, antibodies were infrequently detected. Antibodies were not detected in any of the sera, using qualitative agglutination screening tests with intact blastospores of *C. albicans*.

Mice in the various experimental groups were footpad tested with HEX. Specific data are presented in Fig. 6 from one experiment in which 5×10^5 live and killed blastospores were administered in various sequences. Similar data were obtained, however, when animals were given 1.0×10^6 live or killed blastospores, and the results obtained when mice were footpad tested 2 weeks after one or two cutaneous inoculations with viable inocula have been confirmed several times. The data presented in Fig. 6 are the values resulting from subtraction; i.e., the mean net increase in footpad thickness of control animals has been subtracted from the mean net increase in footpad thickness of the inoculated animals. Mice inoculated cutaneously once with a viable inoculum and footpad tested 14 days later developed small but significant reactions (Fig. 6C), which were sustained through 48 h. Mice inoculated cutaneously twice (Fig. 6A) reacted with a much more pronounced swelling at 24 and 48 h. On the other hand, the inoculation of killed blastospores 2 weeks before testing, after either a prior inoculation with viable blastospores (Fig. 6D) or a prior inoculation with killed blastospores (Fig. 6B), did not stimulate responses significantly greater than those observed in control animals.

Therefore, two cutaneous inoculations with killed blastospores did not induce the formation of delayed responses readily detectable by the footpad swelling test. A single inoculation of killed organisms, however, did seem to prime the animals for an increased footpad responsiveness when they were subsequently inoculated with viable blastospores, since animals given a killed inoculum first and later challenged with a viable inoculum responded with footpad swelling that was similar in magnitude to that of animals receiving two viable inocula.

DISCUSSION

The intracutaneous inoculation of a suitable number of viable blastospores into previously unsensitized animals resulted in the development of a lesion that was confined to the dermis and was of a limited duration, 2 to 3 weeks. The lesions were essentially abscesses containing large concentrations of polymorphonuclear leukocytes. It had been hoped that once an animal

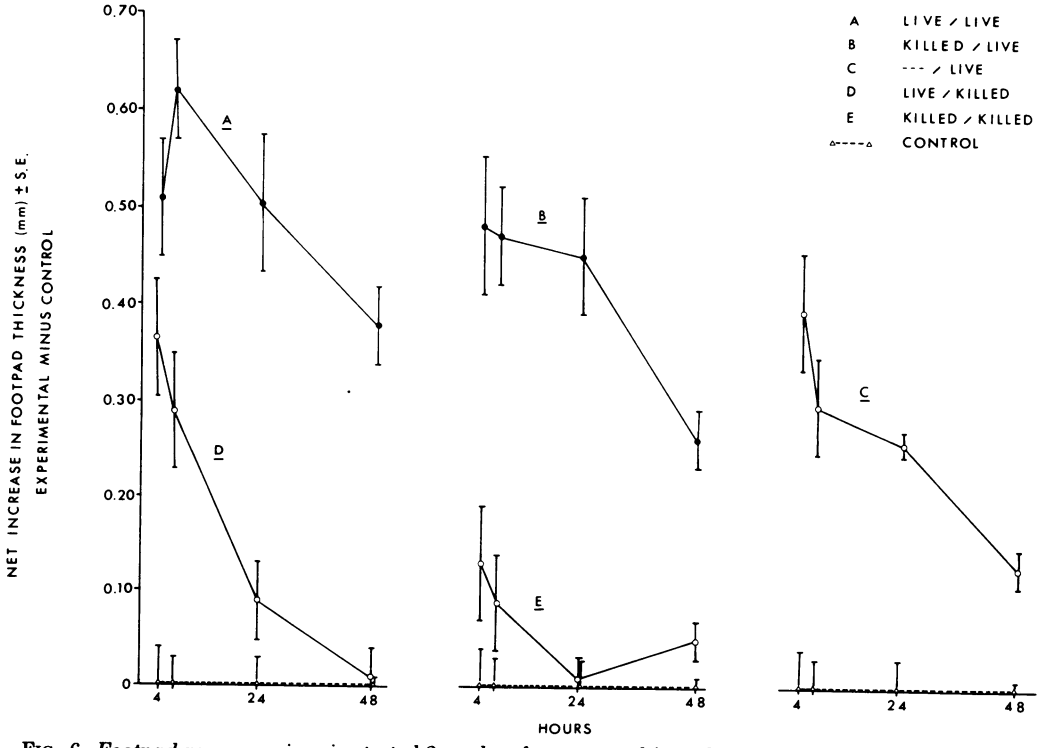

FIG. 6. *Footpad responses in mice tested 2 weeks after a second inoculation with viable or heat-killed C. albicans blastospores (A,B,D,E) or after a single inoculation with viable blastospores (C). n = 20. Mean control values have been subtracted from mean experimental values.*

recovered from a first infection with *Candida*, a protective immune response could be demonstrated by infecting the same animals cutaneously a second time and noting that a lesion evolved which was smaller and/or of shorter duration than that induced by the first infection with a similar dose. Such was not the case; in fact, the host response was more intense and the second lesions were larger than the first.

There are several possible explanations for the increased lesion size when animals were reinfected with *Candida*. For example, the lesions resulting from a second infection could be the result of a nonspecific inflammatory response superimposed on a delayed hypersensitivity reaction, although it appears unlikely that this is the sole explanation, since killed blastospores injected into previously infected mice elicited very small lesions that were not equal to the difference in size between the lesions resulting from first and second exposures to viable *Candida*. One cannot rule out this possibility, however, until it is determined that the process used to kill the blastospores (heating to 60°C for 30 min) did not alter some antigenic component responsible for the delayed hypersensitivity reaction. A second and more likely explanation for the increase in lesion size is related to the observation that, when histological sections of the second lesions were stained with periodic acid-Schiff and examined microscopically, there appeared to be a greater number of intact fungal cells in those lesions resulting from reinfection than in those lesions resulting from a first infection. For some as yet undetermined reason, viable *Candida* introduced dermally into previously infected animals may multiply to a greater extent, and either the presence of increased numbers of blastospores or pseudohyphae themselves resulted in a grossly larger lesion, or the increased number of fungal forms resulted in a greater chemotactic stimulus that attracted more inflammatory cells to the area. Chemotactically active components of *C. albicans* have been described (9, 34).

Using the cutaneous model of infection and reinfection, it was virtually impossible to measure protection, since the pathology in response to reinfection was more rather than less intense, but protective responses were induced because mice infected cutaneously were more resistant to an intravenous challenge than mice that had not been exposed to *Candida* previously. The protection was short-lived, however, if only one cutaneous inoculation was used as the sensitizing infection, in that animals infected once cutaneously 2 weeks before intravenous challenge were protected to varying degrees, but those infected 4 weeks before intravenous challenge were not protected at all. Although the data are not presented here and will be presented in a subsequent communication, two cutaneous infections with viable *Candida* seemed to stimulate a more lasting immunity, because animals challenged intravenously 4 to 5 weeks after the second cutaneous inoculation were protected to the same extent as those challenged intravenously 2 weeks after the second cutaneous inoculation.

Several experimental models of candidiasis in rodents have been proposed in recent years. First, Pearsall and Lagunoff (22) described a mouse thigh model. As with the cutaneous lesions described here, it could be measured and followed with time and was self-limiting. The histopathology of the lesions described was similar to that described by us as well, but no immunological data were presented. Secondly, a cutaneous model was described by Ray and Wuepper (25) wherein *Candida* blastospores were applied to the skin of newborn rats and mice and the area was occluded with a dressing. Again, microabscesses developed with considerable neutrophil accumulation, but immune responses were not evaluated nor was the response to reinfection tested. In fact, most of the attempts to develop models have centered around the establishment of an infection, i.e., colonization or invasion (e.g., 8, 23, 29), and few attempts have been made to assess protection in the system.

There are several reports in the literature, however, of acquired resistance to *C. albicans* induced by viable inocula. Mourad and Friedman (20) reported some protective effect if viable *C. albicans* blastospores were inoculated subcutaneously on six occasions before intravenous challenge. No data were presented on the immune status, e.g., the presence of antibody or delayed-type hypersensitivity, of the animals at the time of the challenge. The large number of organisms inoculated each time, viz., 4×10^7 blastospores, and the multiple doses over a period of 40 days, however, would probably favor antibody production, perhaps to the detriment of delayed hypersensitivity. Hasenclever and Mitchell (14) used a different route of sensitization, intraperitoneal, and a dose of approximately 2×10^7 blastospores was given both 14 and 7 days before intravenous challenge. Experiments were terminated after only 28 days, and cultures of kidneys of surviving animals did not show striking differences between previously infected and control mice, although survival was increased in previously infected animals. Their data, as ours, emphasize the necessity for a viable inoculum, since the same number of nonviable *C. albicans* did not induce a protective

response. Again, the mice were not evaluated for the presence of specific immune responses, i.e., antibodies or cellular immune manifestations.

We believe that this model is suitable for studying immune responses in candidiasis and has several advantages over those previously proposed. The lesion can be observed daily without difficulty and the organisms do not spread systemically, so that the investigator can visualize at all times the stage of infection. Since there does not appear to be systemic spread, the studies could be expanded easily to include in vitro tests of cellular immunity with cells obtained from a variety of sources, e.g., peritoneal cavity, spleen, and peripheral blood. In a subsequent publication, we will attempt to show how the model can be applied to immunologically modified animals.

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