INFECTION AND IMMUNITY, Sept. 1978, p. 729-737
0019-9567/78/0021-0729$02.00/0

Vol. 21, No. 3
Printed in U.S.A.

Experimental Murine Candidiasis: Pathological and Immune Responses in T-Lymphocyte-Depleted Mice

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Received for publication 24 April 1978

Mice depleted of T-lymphocytes by thymectomy and irradiation (TXB) and immunologically competent mice were compared for gross and histological pathology as well as immune responses after cutaneous and/or intravenous challenge with *Candida albicans*. In response to a first cutaneous inoculation with viable *Candida*, TXB, sham-operated (SXB), and unmanipulated (normal) mice, all developed lesions of comparable size, duration, and histopathology. When challenged a second time cutaneously, normal and SXB mice developed lesions which were greatly increased in size when compared with those produced by a first cutaneous infection, whereas TXB mice developed lesions comparable in size to those initiated by the first infection. Histologically, the first and second lesions in all animals were acute abscesses predominantly comprised of polymorphonuclear leukocytes. The larger second lesions in SXB and normal mice were accompanied by detectable circulating antibody and by delayed hypersensitivity. Neither circulating antibody nor delayed hypersensitivity were stimulated in the TXB mice. When challenged intravenously, all previously uninfected mice, regardless of T-cell status, were equally susceptible to *C. albicans*. Contrary to SXB or normal mice, however, TXB mice which had been infected cutaneously were not more resistant to a subsequent intravenous challenge as judged by 6-week survival. The results suggest that T-cells do not play a significant role in innate resistance of mice to systemic candidiasis, but that such cells are important in the development of acquired resistance.

Candida albicans (Robin) Berkhout is a fungus which does not usually invade the normal host, although it is a member of the normal human microbial flora. The mechanism by which healthy individuals resist invasion is unclear, but it is generally believed that cell-mediated immunity, and thus the T-lymphocyte, is an important factor in acquired resistance. The latter statement is based primarily on clinical evidence, in that individuals with immunodeficiency diseases (3, 18, 22, 32, 35) or with diseases for which the treatment itself is immunosuppressive (1, 17, 20, 24, 28, 37) are particularly susceptible. Experimental evidence to corroborate the clinical evidence is somewhat meager. Recently, however, a natural model of immunological modification, viz., the nude or athymic mouse, has been used to investigate innate resistance to *C. albicans*. Cutler (10) inoculated nude mice with large doses of *C. albicans* intravenously and observed that such mice were more resistant to this challenge than normal littermates, and Rogers et al. (30) confirmed the data of Cutler but used smaller challenge doses and compared nude with thymus-implanted nude mice.

In our study, we chose a different model of T-lymphocyte deprivation, viz., mice thymectomized, irradiated, and bone marrow reconstituted as adults, to investigate the interaction between immunologically modified animals and *C. albicans*, and we chose to investigate not only their response to a first exposure to *C. albicans*, but also to reinfection. These studies, in fact, are based on an experimental model of murine candidiasis defined in normal, i.e., immunologically unmodified, mice (13, 14), and involve cutaneous inoculation with viable *C. albicans*, intravenous challenges, and tests for humoral and cellular responses at various times after cutaneous inoculation. In those studies, resistance to reinfection, antibodies, and delayed hypersensitivity were demonstrable after cutaneous inoculation.

MATERIALS AND METHODS

Cultural and cell fractionation procedures. *C. albicans* B311, serotype A, obtained from H. Hasen-

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clever, was maintained by monthly transfer on glucose-peptone agar slants and stored at 4°C. Since specific cultural and fractionation techniques have been reported in detail elsewhere (13, 14), they are described only briefly here. Viable blastospores for inoculation into mice were grown in a soy dialysate broth (27). The antigen used for footpad testing, designated crude HEX, was extracted from a membrane fraction of *C. albicans* by using phosphate-buffered saline (pH 7.4) at 50°C. The protein concentration of the extract was adjusted to 20 μg per test dose. The antigen used in the counterimmunoelectrophoresis assays for the detection of precipitating antibodies was the soluble cytoplasmic substance, i.e., that fraction of the *C. albicans* cell which remains in the supernatant after the final centrifugation (144,000 × *g*) of cells previously broken in a Braun homogenizer.

Preparation of mice. T-depleted mice were prepared by a procedure similar to that described by Davies (12). Male CBA/J mice, 4 weeks of age, were obtained from Jackson Laboratories, Bar Harbor, Me., and their thymuses were removed by suction after the opening of the chest cavity. Additional mice were subjected to identical surgery, but their thymuses were not removed. Two weeks after surgery, both thymectomized and sham-operated mice were irradiated with 850 rads from a ^{60}Co source, 100 rads per min, and within 3 h of the irradiation were reconstituted with 5×10^6 syngenic bone marrow cells. To verify that the dose of irradiation was, in fact, lethal, a few thymectomized and sham-operated mice were always held without bone marrow reconstitution. All mice, including normal controls, were maintained on tetracycline for 14 days after irradiation (125 mg of tetracycline per liter of drinking water) and were rested for a minimum of an additional 2 weeks beyond cessation of antibiotic prophylaxis before the actual experiments were begun.

Immunological evaluation of mice. T- and B-cell levels of normal, SXB, and TXB mice were estimated on the basis of immunofluorescent staining of isolated mononuclear cells from peripheral blood, as well as on a functional basis involving immunization with sheep erythrocytes (SRBC) or lipopolysaccharide (LPS), or by lymphocyte stimulation with mitogens.

For immunofluorescent staining, a total of 3 to 5 ml of peripheral blood with 50 U/ml of heparin (The Upjohn Co., Kalamazoo, Mich.) was collected from 4 to 6 mice of each type, diluted with 3 to 4 volumes of cold RPMI-1640 (Grand Island Biological Co. [GIBCO], Grand Island, N.Y.), and layered onto 3-ml portions of Ficoll-sodium diatrizoate (LSM; Litton Bionetics, Kensington, Md.) After centrifugation at 400 × *g* for 30 min, the buffy layer was collected and washed three times with cold RPMI. For the estimation of theta (Thy-1.2)-positive lymphocytes, mononuclear cells separated on LSM were incubated for 30 min at 4°C with goat anti-rabbit immunoglobulin G labeled with fluorescein isothiocyanate (Cappel Labs, Downingtown, Pa.). The number of cells bearing surface immunoglobulins was estimated by incubating the washed mononuclear cells with rabbit anti-mouse total immunoglobulins labeled with tetramethyl rhodamine isothiocyanate (Cappel Labs.) for 30 min at 4°C, and washing three times with phosphate-buffered saline. Wet mounts of the stained cells were prepared in 7% glycerol–phosphate-buffered saline, and 200 cells were observed and scored for fluorescent patterns by using a Leitz fluorescent microscope equipped with filters BG-12, BG-38, and K510 or K530 for fluorescein, and BG-36, AL-542, and K-570 or K-580 for rhodamine.

To detect the presence of functional T-lymphocytes, three mice each from the normal, SXB, and TXB groups were immunized with SRBC 35 days after the irradiation portion of the mouse preparation procedure. The protocol followed was that published by Jerne et al. (21), wherein the mice were immunized by an intravenous inoculation with 4×10^8 SRBC, and 5 days later their sera, in serial twofold dilutions, were assayed for anti-SRBC antibodies by direct hemagglutination in microtiter plates. The same number and groups of mice were used to detect B-lymphocyte activity. In this case, mice were immunized by intravenous inoculation of 5 μg of *E. coli* 055:B5 LPS (Difco Laboratories, Detroit, Mich.; phenol extraction) in 0.5 ml of 0.15 M NaCl. The protocol followed was that of Benner and Van Oudenaren (2). The LPS used to sensitize SRBC for the hemagglutination assay was detoxified by boiling a solution containing 1 mg of LPS suspended in phosphate-buffered saline (pH 8.0) per ml for 2 h. In both of the hemagglutination assays for T- and B-lymphocyte activity, normal unimmunized mice were bled, and their sera were tested along with sera from immunized animals.

Lymphocyte transformation analyses were performed by using purified phytohemagglutinin (Wellcome Reagents Ltd., Beckenham, England) and LPS in a micromethod (33). Spleens were collected and pressed through 80-mesh stainless-steel screen. After dispersing clumps of cells, erythrocytes were lysed by the addition of hypotonic saline (0.3%, wt/vol; pH 5.75) followed in 30 s by complete medium. The cell suspensions were then adjusted to 2.5×10^6 viable nucleated cells per ml in RPMI-1640 containing 25 mM *N*-2-hydroxyethyl piperazine-*N*-2-ethanesulfonic acid, 2 mM L-glutamine, 100 μg of streptomycin, 100 U of penicillin per ml, and 5% heat-inactivated fetal calf serum. All media components were obtained from GIBCO. Each well in the Microtest II plates (Falcon Plastics, Oxnard, Calif.) received 0.1 ml of cell suspension and 0.1 ml of either complete medium or complete medium containing mitogen. The plates were incubated at 37°C in 5% CO_2–95% air for 48 h, pulsed with 1 μCi of tritiated thymidine (New England Nuclear Corp., Boston, Mass.) for 18 h, and harvested and washed with 0.15 M NaCl on a MASH II unit (Microbiological Associates, Inc., Bethesda, Md.). The filter strips were dried at 100°C for 30 min, and individual discs were punched out into liquid scintillation vials. A 10-ml amount of scintillation cocktail was added to each vial, and the samples were counted in a Beckman LS-250 liquid scintillation counter (Beckman Instruments, Inc., Fullerton, Calif.). The counts obtained were expressed in two ways, mean counts per minute of experimental minus control (E − C) and mean counts per minute of experimental divided by control (E/C).

Evaluation of responses to infection with *C. albicans*. The preparation of inocula, techniques of inoculation, and methods for immunological evalua-

tion after the initiation of infection have been described in detail elsewhere (13, 14). Briefly, animals were infected once or twice cutaneously with 5.0×10^5 viable *C. albicans* blastospores, 2 weeks apart, and subsequently challenged intravenously with 1.0×10^4 viable blastospores. After the cutaneous inoculations and before intravenous challenge, they were tested for delayed hypersensitivity by a footpad swelling technique (8, 9) 13 days after the first or second infection. Feet were measured with dial-type calipers (Schnelltäster, H. Kröplein GMBH, Schluechtern, Hesse, West Germany) before and after inoculation with antigen, and the net increase was determined. Antibodies were assayed by counterimmunoelectrophoresis (15, 26) with a soluble cytoplasmic antigen (14) and by a whole-cell agglutination technique (34, 36).

Histopathology. Randomly chosen mice from each experimental or control group were sacrificed, and either the dermal lesion resulting from the inoculation with viable *C. albicans* blastospores or the antigen-injected foot was removed, fixed in buffered 10% Formalin, embedded in paraffin, sectioned, and stained with hematoxylin-eosin or periodic acid Schiff (16). The sections were coded, and each was examined without knowledge of the experimental group to which the animal had been assigned. Severity of acute and chronic inflammation, the presence of immature lymphoid cells, the approximate number of plasma cells, the approximate number of recognizable fungi, the degree of fibrosis or scarring, and the condition of the overlying epidermis were tabulated. Differences between the groups were evaluated statistically (4).

RESULTS

T- and B-lymphocyte evaluation of mice. The recovery values for mononuclear cells from peripheral blood centrifuged on LSM were somewhat low and ranged from 10 to 40% when total leukocyte counts of samples before separation were compared with numbers of washed mononuclear cells. Despite this fact, it could be concluded from these studies that the TXB mice were, in fact, T-lymphocyte deficient in that they had <1% theta-positive cells, whereas normal and SXB animals had 50 to 60% theta-positive cells. Moreover, there was a relative enrichment of immunoglobulin-positive cells in TXB mice to 40 to 50%, in comparison with 8 to 10% for such cells in normal and SXB mice. No additional studies were done to characterize the remaining cells in the preparations.

When mice were immunized with SRBC or LPS to detect functional T or B cells, respectively, it was apparent that the T-depleted mice had only residual T-lymphocyte activity (Table 1). The hemagglutinating titers to SRBC in T-depleted mice were very low, whereas those in SXB and normal mice were high. TXB mice did have B-lymphocyte function in that they were capable of producing antibodies to LPS although, in general, antibody titers to LPS were somewhat lower in SXB and TXB animals than in normal mice.

TABLE 1. *Hemagglutinating titers in response to immunization with SRBC or LPS in three mice each of normal, sham (SXB), or T-depleted (TXB) animals 35 days after irradiation*

Antigen	Titer[a]		
	Normal	SXB	TXB
SRBC[b]	256	256	4
	512	256	<2
	512	128	<2
LPS[c]	512	64	64
	1,024	256	512
	256	128	64

[a] Titer reported as reciprocal of the highest dilution showing a 2+ agglutination.

[b] Sera from unimmunized mice showed no agglutination with SRBC.

[c] Sera from unimmunized mice showed a slight agglutination with LPS at a 1:2 dilution.

The response of splenic lymphocytes to mitogens, in general, confirmed the immunization data above, although the data are more difficult to interpret. An even wider range of mitogen concentrations was used in early experiments on cells from normal mice, but only those exposed to the optimum range of concentrations are presented here (Table 2). If one compares the net counts, i.e., E − C, resulting from exposure to PHA in experiment 2 for example, those of cells from normal and SXB mice are approximately equal, whereas those of cells from TXB mice are low or negative. However, if these data are converted to stimulation ratios, these E/C values are much higher in normal than in SXB animals. This apparent discrepancy may be attributed to the fact that cells from irradiated animals had a high rate of background DNA synthesis, an observation made in both experiments. In experiment 2, for example, the mean of the unstimulated control values for normal mice was 3,311 cpm, whereas the means for SXB and TXB mice were 21,404 and 20,975 cpm, respectively. Under these conditions, the E/C ratios for cells from SXB and TXB animals in response to LPS would not be considered significant if an E/C ratio of 3.0 were used as the minimum level of significant stimulation. In experiment 1, E/C values for responses to both phytohemagglutinin and LPS were much higher, and cells from normal mice responded to a greater degree than those from SXB mice. Cells from TXB mice responded as well to LPS as did those from SXB mice, however.

Gross pathology and immune responses after intracutaneous inoculation with *C. albicans*. Normal, SXB, and TXB mice were inoculated intracutaneously with 5×10^5 viable *C. albicans* blastospores on day zero, and the

TABLE 2. *Mitogen stimulation of splenic lymphocytes from uninfected normal, sham (SXB), or T-depleted (TXB) mice 35 days or 9 months after irradiation*

Mitogen (μg/well)	Lymphocyte stimulation[a]					
	Normal		SXB		TXB	
	Expt 1	Expt 2	Expt 1	Expt 2	Expt 1	Expt 2
PHA[b]						
0.06	ND[c]	50.7 (21.1)	ND	48.2 (3.6)	ND	0 (<1.0)
0.13	177.9 (47.6)	61.3 (25.4)	97.7 (13.9)	45.0 (3.2)	20.3 (2.4)	2.0 (1.1)
0.25	140.1 (37.8)	60.1 (24.4)	78.4 (11.2)	42.4 (3.1)	15.1 (2.1)	0 (<1.0)
0.50	ND	32.5 (13.6)	ND	22.7 (2.3)	ND	0 (<1.0)
LPS[b]						
6.25	ND	32.7 (13.9)	ND	27.4 (2.3)	ND	11.3 (1.6)
12.50	ND	42.9 (18.8)	ND	30.0 (2.4)	ND	16.3 (1.8)
25.00	115.3 (31.0)	45.8 (19.8)	94.9 (10.8)	37.0 (2.9)	107.2 (7.5)	17.7 (1.9)
50.00	119.1 (32.0)	41.6 (18.9)	87.7 (10.3)	44.1 (3.1)	112.0 (12.1)	17.1 (1.9)

[a] Values indicate counts per minute $\times 10^{-3}$, E − C; values in parentheses indicate stimulation indexes, E/C.
[b] PHA, Phytohemagglutinin; LPS, lipopolysaccharide.
[c] ND, Not done.

lesions that developed were measured daily for 13 days (14). On day 14, each mouse was reinoculated with the same number of viable blastospores as were groups of previously uninfected mice. Therefore, lesion volumes in response to primary and secondary inoculations with viable *Candida* could be compared. All three types of mice responded with lesions of equivalent size when inoculated with blastospores for the first time (Fig. 1). When inoculated a second time, however, the T-depleted mice could be distinguished readily from those with T-lymphocytes, in that normal and SXB mice developed lesions which were much larger the second time, despite the fact that the number of blastospores introduced remained constant. T-depleted mice did not develop larger lesions when reinoculated. The primary and secondary lesions of T-depleted mice were virtually identical.

Delayed hypersensitivity to a *Candida* antigen, crude HEX, could be elicited in normal and SXB mice as well, especially 2 weeks after the second cutaneous inoculation with viable blastospores (Fig. 2). There was a low level of footpad reactivity detected in all groups of animals 2 weeks after the first cutaneous inoculation with *Candida*, but 24-h reactions did not differ significantly from control reactions in TXB mice, whereas they did in normal and SXB animals. A second cutaneous inoculation with viable *Candida* served to boost the delayed hypersensitivity response in normal and SXB mice but had no effect on the response observed in TXB mice. When footpad tissues from mice which had been injected with crude HEX were examined for histological changes, the predominant cell type was the polymorphonuclear leukocyte.

The results of testing sera from different groups of mice by counterimmunoelectrophoresis with a soluble cytoplasmic antigen, are summarized in Table 3. Approximately 6% of normal animals developed precipitating antibodies in response to one cutaneous inoculation with viable *Candida* when tested 2 weeks later. Approximately 50% of normal and SXB animals, however, developed antibodies by 2 weeks after a second cutaneous inoculation, whereas only 7% of the TXB animals had antibodies at that time. No agglutinins were detected in any group of mice.

Histopathology of cutaneous lesions. Cutaneous lesions for histopathological examination were excised from normal mice which had been inoculated once or twice with viable *C. albicans* blastospores, as well as from SXB and TXB mice similarly infected or reinfected. In all animals, an abscess surrounded by a mixed acute and chronic inflammatory reaction dominated the histological picture. Only rarely did a fibrous scar persist. There was no discernible pathological difference between reactions of animals inoculated once and those inoculated twice. Likewise, there was no discernible difference between lesions from thymectomized and nonthymectomized mice. Most of the lesions had concentrations of foamy histocytes, but they were never aggregated to form a granuloma. Giant cells were not observed, but there were well-defined, vascularized aggregates of lymphoid cells in approximately one-third of the animals. These lymphoid aggregates did not occur significantly more frequently in any one group of animals. Overall, the histological changes were consistent with an acute inflammatory response, the predominant cell type being the polymorphonuclear leukocyte.

Intravenous inoculation with viable *C. albicans*. Groups of 15 to 20 normal, SXB, or

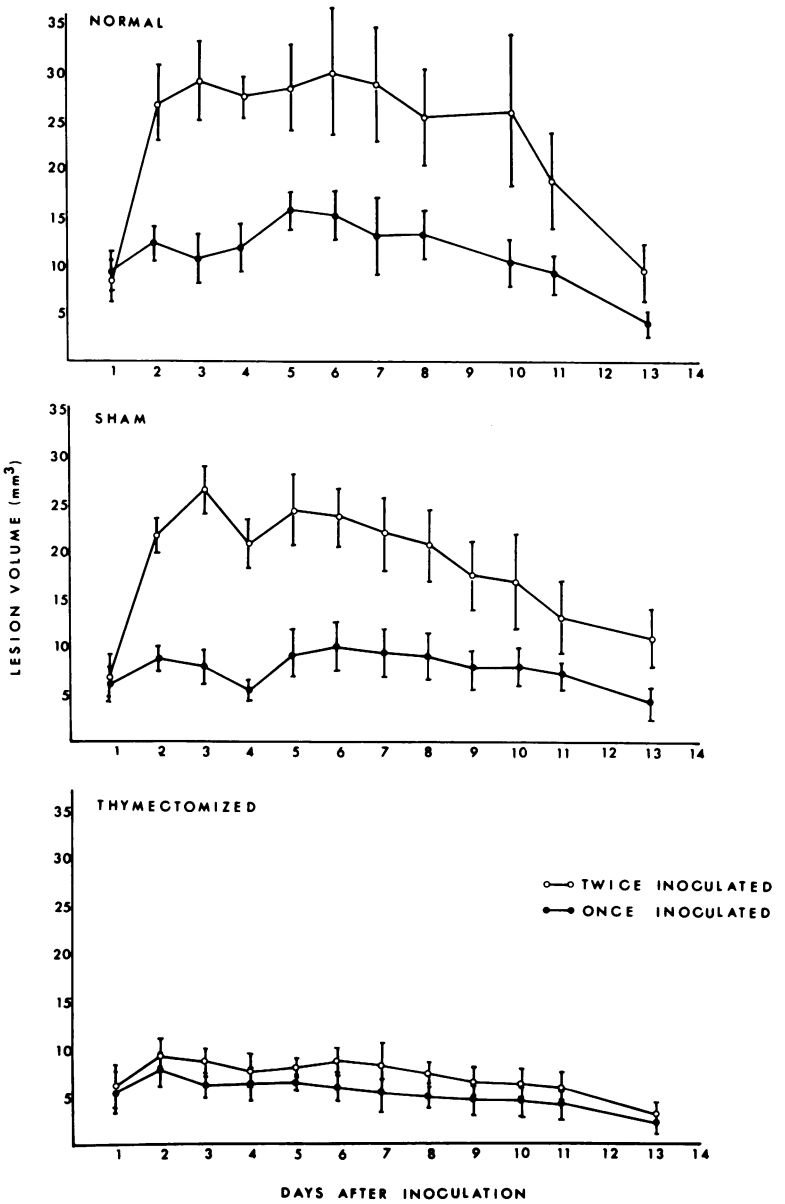

FIG. 1. *Lesion volumes resulting from the cutaneous inoculation of 5.0 × 10^5 C. albicans into normal, sham, or thymectomized mice not previously exposed to C. albicans, or into mice cutaneously infected with the same dose 2 weeks before. n = 20. Bars indicate standard error.*

TXB mice which had been infected cutaneously twice, the second inoculation occurring 3 to 5 weeks previously, along with similar numbers of previously uninfected mice (infection controls) were challenged intravenously with 1.0 × 10^4 viable blastospores to test for evidence of resistance to systemic infection. The data are summarized in Fig. 3 and 4, the latter being a statistical analysis. All of the previously uninfected normal mice died during the course of the experiment, whereas 89% and 76% of the previously uninfected SXB and TXB mice, respectively, died. There was little difference, then, between the rate of death of SXB and of TXB mice that had not been exposed previously to *Candida*. The major difference observed among the three groups was that normal and SXB animals developed a degree of resistance to reinfection after a second cutaneous infection, whereas TXB animals did not. These data have

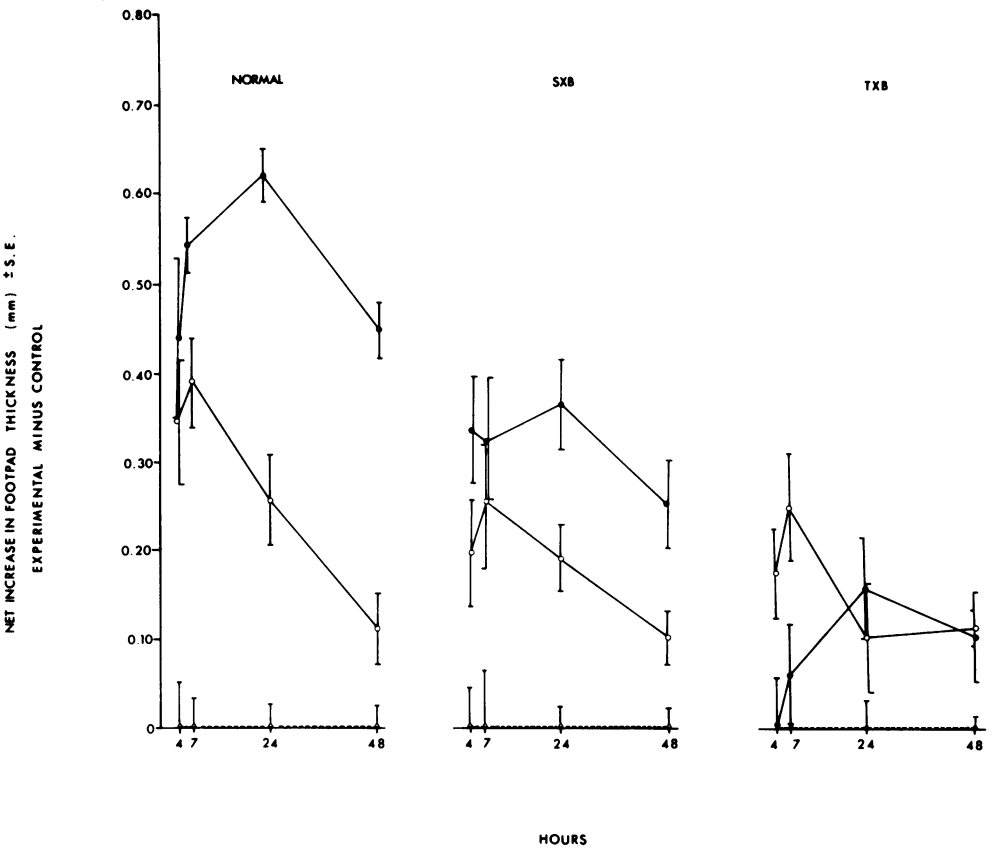

FIG. 2. *Footpad responses to crude HEX (20 μg of protein per test dose) in normal, sham, or thymectomized mice 2 weeks after one* (○) *or two* (●) *cutaneous inoculations with 5.0 × 10^5 viable C. albicans. Footpad responses from uninfected mice* (△) *have been subtracted from those of the infected animals, and the standard error bars for the controls have been placed on the base line. n = 20.*

TABLE 3. *Number of normal, sham, or thymectomized mice in which precipitating antibodies could be detected by counterimmunoelectrophoresis after intracutaneous inoculation with viable C. albicans, using SCS as antigen*

Experimental group	No. of mice with antibodies/total		
	Preinfection	14 Days after first infection	14 Days after reinfection
Normal	0/135	8/135	41/90
Sham-operated	0/30	0/30	13/30
Thymectomized	0/30	0/30	2/30

been analyzed statistically by life table analysis (6, 11; Fig. 4), and significant differences were demonstrable between infection control and twice-infected normal and SXB mice, but not between similar groups of TXB animals.

DISCUSSION

T-lymphocyte-depleted mice prepared by thymectomy and irradiation were neither more susceptible nor significantly more resistant than their sham-operated counterparts to either cutaneous or intravenous challenge with viable *C. albicans* blastospores. Previously uninfected SXB and TXB mice, however, seemed to be somewhat more resistant to an intravenous challenge with viable blastospores than normal mice that had never been exposed to surgery or irradiation. Possibly, this is due to a higher phagocytic index or activation resulting from repair activity occurring in response to the irradiation of the SXB and TXB animals. In fact, the data derived from the cutaneous and systemic challenge of previously uninfected animals in this study would seem to indicate that the innate defense system, unaltered by the depletion of T-lymphocytes, is equally effective in immunologically competent and incompetent animals. Other investigators have emphasized a role for the innate defense system against systemic candidiasis as well (29, 30).

Perhaps the most significant observation re-

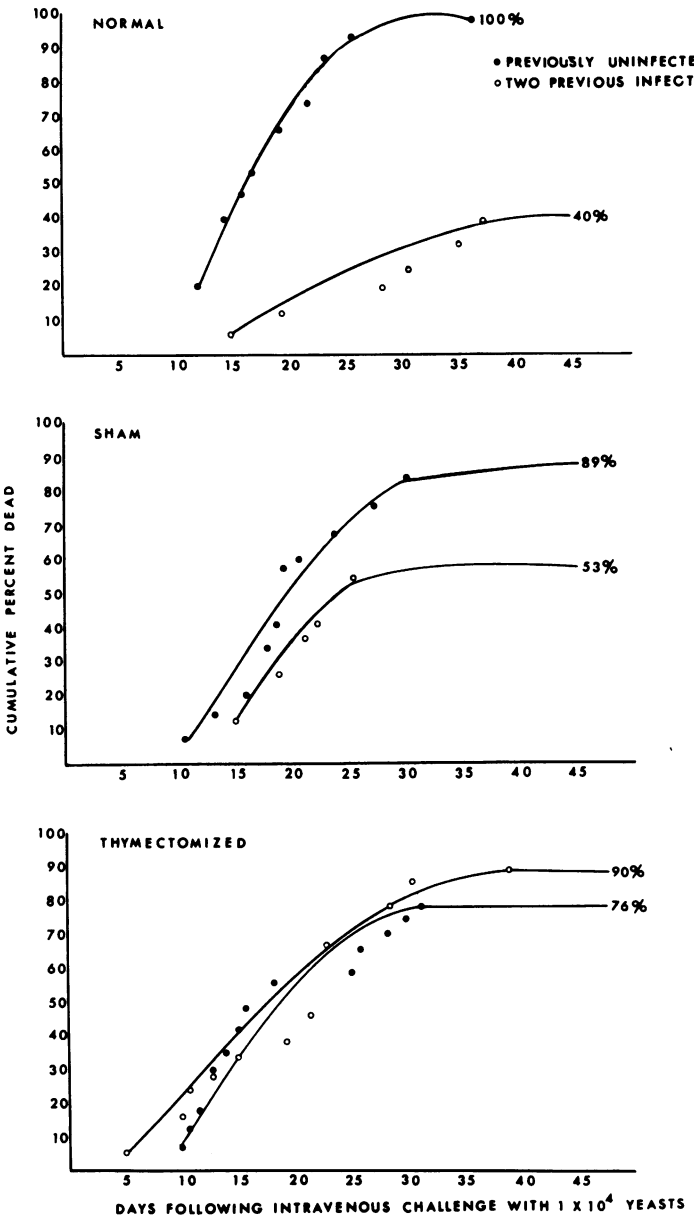

FIG. 3. *Death rates among normal, sham, and thymectomized mice when challenged intravenously with 10^4 viable blastospores 3 to 5 weeks after two cutaneous inoculations with 5.0×10^5 viable blastospores or after no previous infection.*

ported here, however, was that TXB mice developed no immunity (defined here as resistance to systemic challenge) after cutaneous infection and subsequent recovery from that infection, whereas immunity could be demonstrated in both normal and SXB animals. These data are contrary to the findings of Salvin et al. (31), who found neonatally thymectomized mice more susceptible than normal mice to a first inoculation with *Candida*, but are not inconsistent with those of Rogers et al. (30) and Cutler (10), who found congenitally athymic (nude) mice more resistant than their normal littermates to a first infection with *Candida*. None of these groups, however, looked for resistance to reinfection after recovery from a first infection. By using a bacterial "model," Chan et al. (5) found that T-deprived mice were neither more susceptible nor

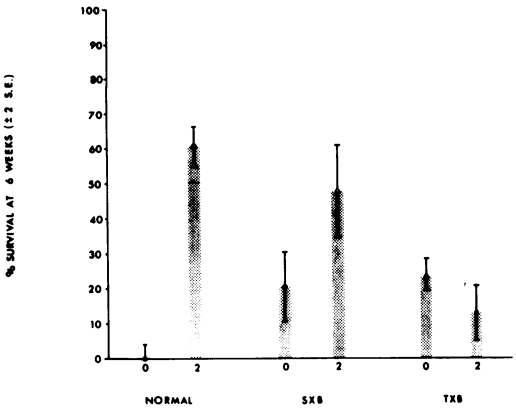

FIG. 4. *Life table analysis of the data presented in Fig. 3. 0, Not previously infected; 2, two previous cutaneous inoculations. Bars indicate standard error. See legend to Fig. 3 for additional details.*

resistant to a first infection with *Listeria monocytogenes*, for which resistance to reinfection has been shown to be due to cellular immunity.

The presence or absence of functional T-lymphocytes also resulted in a rather curious but unresolved observation, viz., those lesions developing in immunologically competent animals which had been exposed previously to *C. albicans* were much larger than the lesion resulting from a first infection with the same dose of organisms, whereas second lesions developing in T-depleted mice were indistinguishable from the primary lesion. Initially, this was thought to be a delayed hypersensitivity reaction superimposed on the normal inflammatory response to the organism. Arguing against this hypothesis is the fact that inoculation with the same number of killed cells does not elicit a lesion in previously infected normal animals (14). It may be, however, that in the process of killing the cells the eliciting antigen is destroyed, or is one that is produced and/or excreted by the metabolically active fungal cell. In normal animals, the peak of delayed hypersensitivity occurs 7 days after a second cutaneous inoculation with viable *Candida* (13), and it is after this time that the lesion begins to wane. The fact that the maximum lesion volume is reached in less than 7 days may simply mean that the dermal response to a product of the viable cell is a more sensitive indicator of delayed hypersensitivity than the footpad assay. Related to this is the fact that the TXB animals did not develop delayed hypersensitivity demonstrable by footpad testing. A somewhat more purified form of antigen used in these studies, HEX, has been shown to detect true delayed hypersensitivity in that the footpad response to it was transferrable with cells and not serum from previously infected animals (13). The histological response of the footpad reaction was not the typical delayed type, i.e., perivascular monocytic infiltrate, but Cohen (7) has pointed out that, in mice, the delayed hypersensitivity reaction contains large numbers of polymorphonuclear leukocytes.

It had been hoped that histological comparisons between the normal and T-depleted animals would result in some clue to the reason for the difference between the two lesions, but the primary and secondary lesions were qualitatively similar; they differed only in intensity. The larger lesion might, in fact, be due to specific antibody, although that idea could be argued against on the basis of the observation (unpublished data) that antibody reaches detectable levels only toward the end of the 2-week period immediately following the second cutaneous inoculation, a time at which the lesion is resolving, not increasing. Kirkpatrick and Smith (23), in trying to explain the clinical manifestations of chronic mucocutaneous candidiasis, a syndrome associated with T-lymphocyte deficiency, have suggested that cellular immunity may be effective in preventing disseminated candidiasis, whereas antibodies may correlate with the severity of the cutaneous manifestations. The manner in which anti-*Candida* antibodies could function is speculative, of course, but Valdimarsson et al. (35) have suggested that antibody may suppress T-lymphocyte activity.

In summary, T-lymphocyte-depleted mice are not more susceptible than immunologically competent mice to cutaneous or systemic candidiasis, nor do they develop acquired immunity, since they do not develop resistance to systemic challenge after recovery from cutaneous infection. The reason for the lack of development of resistance obviously implicates T-lymphocytes, but whether this result is due to the role of the T-lymphocyte as helper in antibody production or is due to an effector cellular immune function per se cannot be answered at this time. In our opinion, however, the model presented here, including the cutaneous inoculations, will be an excellent system in which to study the adoptive transfer which may eventually resolve this issue.

ACKNOWLEDGMENTS

This investigation was supported by Public Health Service grants AI-12308 and AI-00003 from the National Institute of Allergy and Infectious Diseases. D.K.G. and S.A.M. were both trainees in medical mycology.

J.E.D. also acknowledges help and encouragement from Dudley Dumonde, and support from the Wellcome Trust and Research Corporation, which provided the means by which preliminary investigations were carried out. The technical assistance of C. S. Butler is gratefully acknowledged.

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