In vivo effect of mouse peritoneal cells on Candida albicans
by Anne Holly Poor

A thesis submitted in partial fulfillment of the requirements for the degree of MASTER OF SCIENCE
I n Microbiology
Montana State University

Abstract:
Millipore diffusion chambers containing the fungus Candida albicans were implanted in the peritoneal cavity of normal and nude BALB/c mice to study the effects of host soluble and cellular factors on the growth of C. albicans. Opsonins, 51Cr solution, and 51c r-labeled neutrophils were able to enter chambers which were implanted in the peritoneal cavity 2k hr earlier but were unable to enter chambers which were present in the peritoneal cavity for 7 days. Fungal growth in vivo was always greater in chambers that admitted soluble factors but excluded host cells (small porosity chambers) than in chambers that admitted soluble factors and also allowed emigration of host cells; however growth seen in chambers in vitro was several orders of magnitude greater than growth seen in vivo. Peritoneal exudate cells (PEC) were added directly to small porosity chambers with C. albicans to study the effects of specific cell populations on the fungus. Neutrophi 1-rich PEC harvested from normal mice were candidacidal at high neutrophil to yeast ratios, while macrophage-rich PEC were candidastatic at all macrophage to yeast ratios tested. Neutrophi 1-rich and macrophage-rich PEC harvested from nude mice both were candidacidal at high phagocyte to yeast ratios.

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Date Aug. 20, 1979

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by

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TABLE OF CONTENTS

	Page
VITA	ii
ACKNOWLEDGMENTS	iii
TABLE OF CONTENTS	iv
LIST OF TABLES	vi
LIST OF FIGURES	vii
ABSTRACT	viii
INTRODUCTION	1
MATERIALS AND METHODS	8
Source of experimental animals	8
Yeasts and growth conditions	8
Construction of diffusion	9
Filling and implantation of chambers	9
Harvest of chamber contents and assay for viable *C. albicans*	10
Preparation of peritoneal exudate cells (PEC)	10
Serum source	12
Preparation and use of ^{51}Cr labeled cells	12
Phagocytic cell function tests	13
RESULTS	15
Fate of C. albicans in diffusion chambers *in vivo* and *in vitro*	15

Page

Treatment of the fibrin clot inside chambers 20

Emigration of host peritoneal cells into 3p chambers 20

Entry of opsonic factors into diffusion chambers 24

Effect of neutrophil-rich PEC on C. albicans in diffusion chambers 25

Effect of macrophage-rich PEC on C. albicans in diffusion chambers 29

DISCUSSION 33

LITERATURE CITED 44

APPENDIX I Effect of neutrophils on Streptococcus pneumoniae in diffusion chambers 52

APPENDIX II Effect of silica on the phagocytic ability of mice 56

APPENDIX III Effect of phagocytic cells from normal mice on C. albicans in diffusion chambers in the presence of immune serum 61

REFERENCES TO APPENDICES 64

LIST OF TABLES

Table		Page
1.	Growth of C. albicans in diffusion chambers in vitro at 37°C	19
2.	Effect of homogenizing filters and chamber contents on the viability of C. albicans in diffusion chambers	21
3.	In vivo opsonization of C. albicans in diffusion chambers	26
4.	Effect of neutrophils harvested from normal mice on C. albicans in diffusion chambers	27
5.	Effect of neutrophils harvested from nude mice on C. albicans in diffusion chambers	28
6.	Effect of macrophages from normal mice on C. albicans in diffusion chambers	30
7.	Effect of macrophages harvested from nude mice on C. albicans in diffusion chambers	32
8.	Effect of neutrophils on Streptococcus pneumoniae in diffusion chambers	54
9.	Clearance of Candida albicans from blood of normal and silica treated BALB/c mice	59
10.	Effect of silica on the phagocytic ability of BALB/c mice	60
11.	Effect of phagocytic cells from normal mice on C. albicans in diffusion chambers in the presence of immune serum	63

LIST OF FIGURES

Figure		Page
1.	Fate of C. albicans in 0.45p and 3p diffusion chambers *in vivo*	17
2.	Emigration of host peritoneal cells into 3p diffusion chambers	23

ABSTRACT

Millipore diffusion chambers containing the fungus Candida albicans were implanted in the peritoneal cavity of normal and nude BALB/c mice to study the effects of host soluble and cellular factors on the growth of C. albicans. Opsonins, ^{51}Cr in solution, and ^{51}Cr-labeled neutrophils were able to enter chambers which were implanted in the peritoneal cavity 24 hr earlier but were unable to enter chambers which were present in the peritoneal cavity for 7 days. Fungal growth in vivo was always greater in chambers that admitted soluble factors but excluded host cells (small porosity chambers) than in chambers that admitted soluble factors and also allowed emigration of host cells; however growth seen in chambers in vitro was several orders of magnitude greater than growth seen in vivo. Peritoneal exudate cells (PEC) were added directly to small porosity chambers with C. albicans to study the effects of specific cell populations on the fungus. Neutrophil-rich PEC harvested from normal mice were candidacidal at high neutrophil to yeast ratios, while macrophage-rich PEC were candidastatic at all macrophage to yeast ratios tested. Neutrophil-rich and macrophage-rich PEC harvested from nude mice both were candidacidal at high phagocyte to yeast ratios.

INTRODUCTION

Candida albicans is a ubiquitous yeast-like fungus which is the most common etiologic agent of the disease candidiasis. This organism is considered to be an opportunistic pathogen because individuals who get candidiasis are compromised in defense against infection. Candidiasis is a complex disease: it can occur in the human at many sites depending on the severity of the underlying predisposing disorder. The prognosis and therapy of candidiasis varies depending on the site of infection.

Human candidiasis can present in many forms. One form of candidiasis is a simple cutaneous infection. The predisposing factor is generally an abnormality that results in a local loss of innate defense, such as in burns or as in the neonate's lack of normal flora. This form of candidiasis usually improves when the predisposing factor is eliminated, otherwise topical antibiotic therapy may be necessary. Chronic mucocutaneous candidiasis (CMCC) is an uncommon disease which is characterized by a widespread infection of skin, nails, and mucous membranes. Patients with this disease usually have an underlying defect in T cell function. Intravenous therapy with the antimycotic amphotericin B provides only transient remission. Systemic candidiasis has become recognized as a common nosocomial infection; it occurs in patients who are severely debilitated for example by therapeutic immunosuppression, neoplasia, or treatment with broad spectrum anti-

biotics. (21). In systemic candidiasis, it is thought that the fungus disseminates hematogenously from a portal of entry, which is most often the gastrointestinal tract (33,23) but may also be the lung (48). Foci of infection can be seen in different internal organs in a single patient. The lung, liver, spleen, or brain may be affected, however the kidney is the organ most commonly affected. Amphotericin B therapy is essential for recovery from systemic candidiasis.

Clinical observations and experimental animal models point to several aspects of immunity which are important in resistance to candidiasis. Human clinical cases exist which have brought some investigators to suggest that T cell dependent cell mediated immunity (CMI) is important in defense against candidiasis. Individuals with congenital thymic aplasia, such as DiGeorge and Nezelof-Allibone syndromes are more susceptible than normal people to CMCC. Other individuals who have CMCC also exhibit abnormalities in T cell related functions, for example cutaneous anergy to Candida antigen, deficient in vitro lymphocytic transformation responses, or deficient synthesis of macrophage inhibition factor (MIF) after exposure to Candida antigen (21,33,64).

Experimental animal correlates of human disease which also suggest that CMI is important in resistance to candidiasis include a murine model of cutaneous candidiasis and NZB mice. Domer, et al.

found that delayed-type hypersensitivity to cell wall and cell membrane antigens develops after cutaneous infection in mice (20). Other workers found that NZB mice, known to develop abnormalities in T cell dependent function with age, are more susceptible to an intravenous dose of C. albicans than are other strains of mice tested (13).

In addition to studies which suggest that T cells are important in host defense to candidiasis, evidence exists which suggests that phagocytic cells are also important. Biopsy material from human lesions may show an acute inflammatory response surrounding foci of Candida infection, a response consisting predominantly of neutrophils (43). In patients with Candida meningitis an intense pleocytosis of the spinal fluid has been noted, which is like a pyogenic response to bacterial infections (66). The attraction of neutrophils to an area of Candida infection may occur due to generation of chemotactic factors either by activation of the alternative pathway of complement by Candida cell walls (52), or possibly, by metabolic products of the fungus (15). Defective leukocyte chemotaxis is seen in patients with severe burns, this correlates with their increased susceptibility to Candida infections (65). The importance of neutrophils in prevention of dissemination of Candida is suggested in clinical studies which show that granulocytopenia is a major predisposing factor leading to Candida sepsis (45,51). Humans with Chediak-Higashi syndrome have

neutrophils that display abnormal morphology, defective migration, and defective chemotaxis (67). Although these patients usually suffer from pyogenic infections but not candidiasis, a murine correlate of human Chediak-Higashi syndrome (26) exhibits an increased mortality rate over normal mice when infected with C. albicans (22).

In vitro studies form a number of laboratories have substantiated the ability of human neutrophils and macrophages to phagocytize and kill C. albicans (47,37,4). Women who suffered recurrent attacks of vaginal candidiasis had leukocytes that displayed normal ingestion but deficient killing of C. albicans in vitro (58). A previously healthy young girl with disseminated candidiasis had a leukocyte defect that was specific for intracellular killing of C. albicans (61). In one study (64), 40% of CMCC patients studied had normal CMI; one may speculate that these patients had CMCC because of defects in innate immunity. Clinical studies which support this speculation include in vitro experiments where granulocytes from CMCC patients had phagocytic and candidacidal defects (19), and other studies where mononuclear cells from a CMCC patient had defective chemotactic responses (60). In vitro studies by Lehrer and Cline (34-36) demonstrated that leukocytes from chronic granulomatous disease patients and from patients with hereditary myeloperoxidase deficiency were unable to kill C. albicans, indicating that myeloperoxidase and H_2O_2 are necessary in the candidacidal mechanism.

Other experimental animal models exist which suggest that innate immunity is important in defense against candidiasis. Thymectomized mice (29) and congenitally athymic (nude) mice (14,54) are more resistant to certain forms of acute infections by C. albicans. In murine models of cutaneous candidiasis abscesses develop which contain high numbers of neutrophils (28,49). In one study, recovery from candidiasis and resistance to reinfection by C. albicans occurred without histopathological indicators of CMI (49). Corticosteroid treatment of mice was found to potentiate candidiasis and renal Candida lesions in these mice were deficient in inflammatory cells (7). In another study, reduced accumulation of neutrophils at foci of Candida infection was correlated with increased susceptibility of mice to candidiasis when mice were previously injected with L1210 leukemia cells (32). Other workers studied the resistance to candidiasis in experimental animals depleted of certain chemotactic factors. Guinea pigs that were treated with cobra venom factor (CVF) to deplete C3 (53) exhibited increased mortaliity when challenged with C. albicans as compared to control animals which were not treated with CVF (27).

Evidence cited in the above paragraphs indicates that innate immunity plays a role in defense against candidiasis. Other workers have investigated the effect of human (34,37,47) and animal (4) phagocytic cells on C. albicans in vitro. Since results of in vitro

systems can not always be readily interpreted to apply to *in vivo* situations, the goal of this project was to study the effect of phagocytic cells on *C. albicans in vivo*. This work involved implanting Millipore diffusion chambers containing *C. albicans* in the peritoneal cavity of mice to study the candidacidal effects of phagocytic cells *in vivo*.

Diffusion chambers implanted in the peritoneal cavity of mice represent a controllable *in vivo* environment in which cells and cell-host interactions may be studied. Diffusion chambers have been used *in vivo* in the past to study tumor cell growth (1) and host immunity to tumors (2). More recently, diffusion chambers have been used *in vivo* to culture mouse (6,63,11,10,5,9) and human (24,31,8) hematopoeitic cells, to study the regulation (56,52) and kinetics (46) of antibody synthesis by lymphoid cells, and to study the effects of anticancer drugs on various human tumors (40).

In my experiments, *C. albicans* was added to diffusion chambers which were then implanted in the peritoneal cavity of mice. The porosity of the membrane filters composing the sides of the chambers was such that the fungal cells could not escape the chambers. The effect of soluble factors and emigrating peritoneal cells on *C. albicans* in diffusion chambers was examined by constructing the chambers with membrane filters of various porosities. Also, the

effect of specific phagocytic cell populations was examined by adding peritoneal cells directly to chambers with C. albicans. Chambers could then be later removed from the peritoneal cavity and assayed for viable C. albicans.

MATERIALS AND METHODS

Source of experimental animals. BALB/c mice, originally obtained from Baylor Medical School (Houston, TX), were maintained on food (Wayne Lab Blox Allied Mills Inc., Chicago, IL) and acidified water (41) ad libidum. Cages and bedding were sterilized prior to use. Fecal specimens periodically plated onto blood agar, sabouraud-dextrose agar and Mycosel agar (BBL, Cockeysville, MD) were negative for Candida albicans. Sera obtained from randomly selected mice were negative for Candida agglutinins and precipitins (14).

Congenitally thymus-deficient (nude) mice were raised together with their phenotypically normal littermates (NLM; +/+, or nu/+) in separate quarters. Nude mice were from a colony in which cross-inter-cross mating is in progress to derive a line congenic with BALB/c mice. Hereafter BALB/c mice and NLM are designated as normal mice.

Yeasts and growth conditions. Candida albicans strain 9938 (Medical Mycology Unit, Tulane University) was used in all experiments. The yeasts were grown aerobically in 2.0% glucose-0.3% yeast estract-1.0% peptone broth. The cultures were rotated at 160 rpm (Gyrotatory Incubator-Shaker, New Brunswick, NJ) for 48-72 hr at 37°C. Organisms were harvested by centrifugation, washed three times in sterile saline, and resuspended to the appropriate concentration in 0.01M phosphate buffered saline, pH 7.2 (PBS). Yeast viability was determined to be at least 95% by plating suspensions of known concentration onto

sabouraud-dextrose agar.

Construction of diffusion chambers. Millipore membrane filters (13 mm diameter), type HA of 0.45 µm porosity (0.45 p), or type SS of 3 µm porosity (3 p) (Millipore Corp., New Bedford, MA) were glued (Millipore Cement #1) to each side of 3 mm thick Millipore lucite rings and the glue was allowed to dry for at least 8 hr before use at 23-25°C. Some chambers were checked for the presence of leaks by blowing air into chambers under water as advocated by Amos (3). The chambers were surface decontaminated by exposure of each side to uv irradiation for 20 min in a safety-hood (Bioquest, Cockeysville, MD); these conditions were previously determined to be microbicidal.

Filling and implantation of chambers. Chambers were placed between the tips of goose-neck forceps, and 0.15 ml of the cell suspension (either C. albicans alone or C. albicans together with mouse peritoneal cells) was added to each chamber with a syringe and 25 gauge needle through a radially located hole in the lucite ring. The hole was sealed with a small amount of parafin (Sherwood Medical Industries, St. Louis, MO). Normal or nude mice were anesthetized with 0.06 mg/g body weight sodium pentobarbital (50) and the chambers were implanted into the peritoneal cavity through a ventral near-midline incision which was then closed with surgical clips (Autoclips, Clay Adams,

Parsippany, NJ).

Harvest of chamber contents and assay for viable C. albicans. Mice were killed by cervical dislocation and the chambers were removed and added to sterile distilled water for 5 min. The filters were cut from the lucite rings with a scalpel and homogenized with the chamber contents and 5 ml of sterile saline in a glass tissue homogenizer. Dilutions of the homogenates were plated on sabouraud-dextrose agar containing 50 units/ml penicillin and 50 µg/ml streptomycin (Microbiological Associates, Bethesda, MD). Colony forming units (cfu) of C. albicans were counted after 36 hr incubation at 37°C.

Preparation of peritoneal exudate cells (PEC). A neutrophile-rich PEC population was obtained from 12-24 week old male or female normal or nude mice. Mice were injected intraperitoneally (ip) with 3 ml of 0.5% glycogen (Nutritional Biochemicals, Cleveland, OH) in saline. Four to six hours later the mice were killed by cervical dislocation, the abdominal skin was reflected and a small incision was made in the peritoneal wall. The peritoneal cavity was washed out with approximately 5 ml of Hank's Balanced Salts Solution (HBSS) (GIBCO, Grand Island, NY) containing 0.002M disodium ethylene-diamine-tetraacetate (EDTA). The washes were centrifuged at 200 x g for 10 min, the cells were washed once with HBSS, and resuspended in PBS to the appropriate

concentration. The cell population was at least 85% neutrophils as determined by examination of acetic acid wet mounts, which cleared the cells so that the nuclear morphology was evident, and by Giemsa stained smears. At least 90% of the cells were viable as determined by trypan blue exclusion.

A macrophage-rich PEC population was obtained by injecting normal or nude mice ip with 3 ml of NIH thioglycolate (Difco, Detroit, MI). The PEC were harvested 3 days later and cell suspensions were prepared as described above. The cell population was 60-80% macrophages as determined by examination of Giemsa-stained smears, and at least 85% of the cells were viable.

PEC collected from thioglycolate-stimulated BALB/c mice were further enriched for macrophages by ficoll density gradient centrifugation using a method described by Zambala and Asherson (69). About 10^8 peritoneal cells were suspended in 4 ml of 25% ficoll (Pharmacia, Uppsala, Sweden) in Balanced Salts Solution (BSS) (42) and placed in a 30 ml thick-walled glass centrifuge tube (Corning, Corning, NY). The cells were overlaid with 6 ml of 21%, 5 ml of 16% and 4 ml of 12% ficoll solution and centrifuged in a swinging bucket rotor (Sorvall HB-4) at 20,000 x g for 70 min at 4^oC. The cells in the top 2 interfaces were collected with a Pasteur pipette, washed twice with BSS, and suspended to the proper concentration in PBS.

Serum source. In some experiments, the peritoneal cells were suspended in homologous serum rather than PBS. Normal BALB/c mice were lightly anesthesized with ether and bled from the orbital venous plexus, the blood was allowed to clot at 4°C. The serum was used within 2 hr or stored at -20°C until use.

Preparation and use of ^{51}Cr labeled cells. Sodium chromate (^{51}Cr) (New England Nuclear, Boston, MA) of specific activity 300-500 mCi/mg Cr was used to label neutrophil and spleen cell populations using a method described by others (25). Neutrophil-rich PEC were obtained as described above. Spleen cells were obtained from normal BALB/c mice. The mice were killed by cervical dislocation, an incision was made through the peritoneal wall, and the spleen was removed and added to 5 ml of HBSS+EDTA. A single cell suspension was made by gently rubbing the spleen against a small gauge aluminum screen. The suspension was centrifuged at 200 x g for 10 min, and the cells were washed once with PBS. The cells were determined to be at least 80% viable by trypan blue exclusion. The neutrophil and spleen cell populations were suspended to about 2 x 10^8 cells/ml in PBS containing 15% fetal calf serum (FCS) (Microbiological Associates) and 50-75 mCi of ^{51}Cr were added per ml cell suspension. The cells were incubated at 37°C in a humidified chamber under 5% CO_2 for 1 hr, mixed every 15 min, then collected by centrifugation at 200 x g for 10 min and washed

twice with PBS. Approximately 1 x 10^8 labeled PEC or spleen cells were injected intravenously (iv) into normal mice implanted with 3 p chambers containing 5 x 10^4 C. albicans. Chambers were harvested 6 hr after the iv injection of ^{51}Cr labeled cells and rinsed in PBS. Filters and chamber contents were counted separately for 1 min each in a gamma counter (Beckman Biogamma, Beckman Instruments, Fullerton, CA), at window settings of 0 (lower) and 300 (upper). The counting efficiency of this machine was 72% under these conditions. In some experiments, the chamber contents were centrifuged at 200 x g for 10 min and the supernatent liquid and cell pellet were counted separately.

Phagocytic cell function tests. An in vitro phagocytic assay described by others (44) was used to test phagocytic functions of peritoneal cells. Chambers of 0.45 μm porosity containing 1 x 10^8 heat killed yeasts were harvested 24 hr after implantation; the yeasts were recovered by vortexing the filters and chamber contents. PEC were collected from unstimulated normal mice and suspended in medium 199 (M199) (GIBCO); 0.5 ml of this suspension (2 x 10^6 cells/ml), with 5% NMS or NMS absorbed 3 times with 1/10 volume of C. albicans cells at 37°C (INMS) was added to 22 mm x 22 mm cover glasses (VWR Scientific, Seattle, WA) and incubated in a humidified chamber at 37°C for 1 hr. The cover glasses were rinsed with 2 ml of HBSS. One-half ml of the C. albicans suspension obtained from the chambers or 0.5 ml of a heat

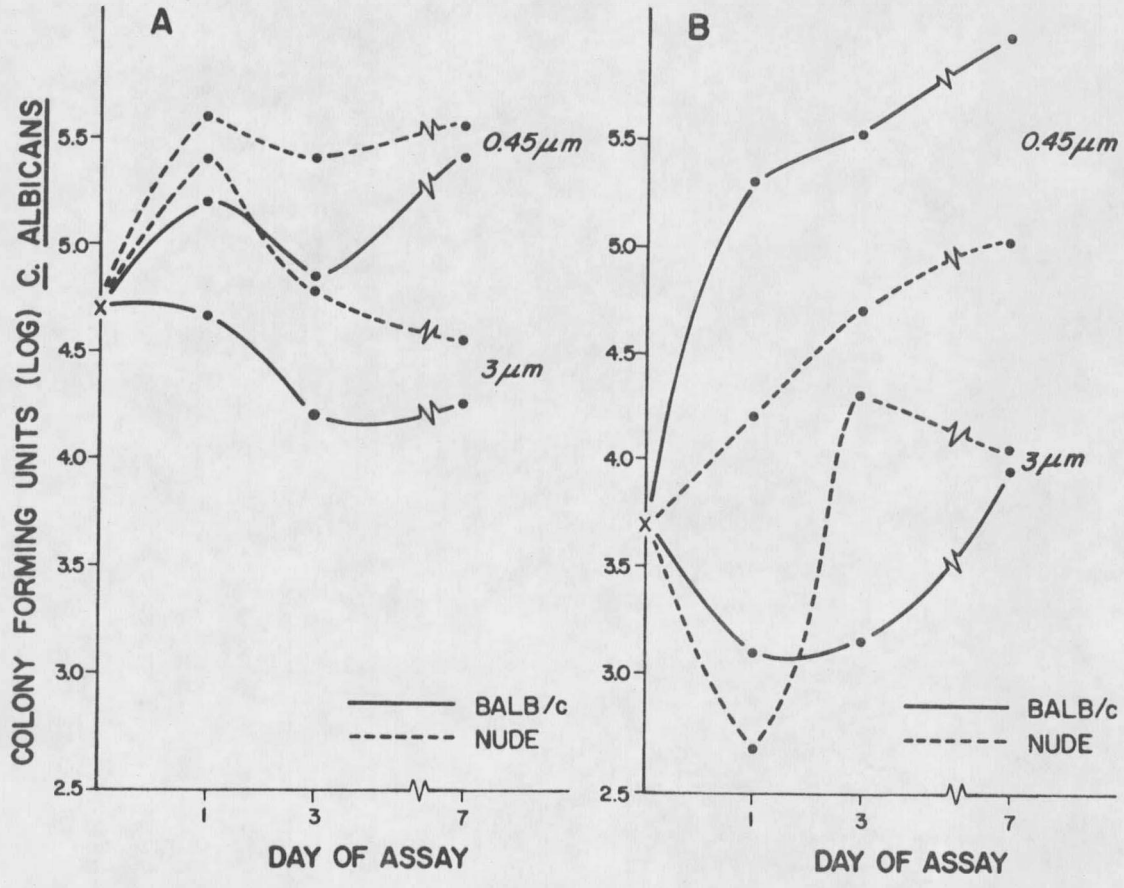
killed C. albicans suspension containing 4 x 10^6 yeast cells per ml with 5% NMS or INMS was added to the cover glasses containing the macrophages and incubated at 37°C for 1 hr. The cover glasses were washed with 2 ml of HBSS, air dried, fixed with methanol for 3 min, stained with Giemsa, and examined using light microscopy at 1000X. All tests were done in duplicate and 200 macrophages per cover glass were counted. The percent phagocytosis represents the mean percentage of macrophages that had ingested one or more yeast cells.

Random migration of neutrophils was studied using the chemotaxis under agarose method (15).

RESULTS

Fate of C. albicans in diffusion chambers in vivo and in vitro. The fate of Candida albicans in vivo in the peritoneal cavity of mice was tested in 3p chambers, which allow emigration of host peritoneal cells, and in 0.45p chambers, which exclude host peritoneal cells. Various concentrations of yeast cells were added to chambers, the chambers were implanted ip into BALB/c or nude mice, and the number of viable C. albicans remaining in the chambers was assayed on days 1, 3, 5, and 7 post-implantation. When 5×10^4 yeasts were added to chambers which were implanted in BALB/c mice (Figure 1-A), the number of fungal units recovered from 0.45p chambers increased by about 0.75 $\log_{10}$ after 7 days of in vivo inclubation, while the number of fungal units recovered from 3p chambers decreased by 0.5 log after 7 days. Similar results were seen when chambers containing 5×10^4 yeasts were implanted in nude mice, except that the number of cfu recovered from 3p chambers at 7 days was approximately the same as the inoculum. Also, the number of C. albicans cfu recovered from both 0.45p and 3p chambers after 28 days of in vivo incubation was about the same as the number of cfu recovered from chambers at 7 days. When 5×10^3 yeasts were added to chambers (Figure 1-B) the number of cfu recovered from 0.45p chambers implanted in BALB/c mice increased over the inoculum by over 2 logs after 7 days, but increased by only about 1 log in 0.45p chambers implanted in nude mice. In contrast, C. albicans cfu recovered from 3p

Figure 1. Fate of *C. albicans* in 0.45p and 3p diffusion chambers *in vivo*. A - 5×10^4 *C. albicans* were added to chambers; B - 5×10^3 *C. albicans* were added to chambers.


A
B
COLONY FORMING UNITS (LOG) C. ALBICANS
0.45μm
3μm
BALB/c
NUDE
5.5
5.0
4.5
4.0
3.5
3.0
2.5
1
3
7
DAY OF ASSAY

chambers implanted in BALB/c mice decreased about 0.5 log after 1 day of *in vivo* incubation, and decreased about 1 log in nude mice. After 7 days of incubation, the number of fungal units recovered from 3p chambers increased to slightly exceed the inoculum.

The growth of *C. albicans* in diffusion chambers *in vitro* was determined by adding various concentrations of yeasts to 0.45p chambers, adding the chambers to 50 ml flasks containing 10 ml of GYEP, and incubating for 48 hr at 37°C. After incubation the filters and chamber contents were removed and either vortexed or homogenized in 5 ml of saline. Regardless of the size of the yeast inoculum and method of recovery (vortexing or homogenizing) the number of cfu recovered from the chambers was approximately the same (Table 1). Similar numbers of yeasts were recovered if the chambers were incubated in 10 ml of FCS rather than GYEP. To determine the accuracy of homogenizing and subsequent plating of filters and chamber contents, 0.45p chambers containing 4×10^7 yeasts were implanted ip and harvested 48 hr later. After vortexing the filters and chamber contents in 5 ml saline, an aliquot of the supernatent liquid was assayed for the number of yeasts by direct counting using a hemacytometer and by plating onto sabouraud-dextrose agar. The same filters and chamber contents were then homogenized and plated onto sabouraud-dextrose agar. Homogenization did not decrease the viability of *C. albicans*, and plate counts of yeasts

Table 1. Growth of C. albicans in diffusion chambers in vitro at 37°C

No. yeasts added	Log_{10} cfu recovered after 48 hr	
	homogenized	vortexed
$4x10^7$	8.34 ± 0.17	8.26 ± 0.14
$5x10^4$	8.29 ± 0.26	8.29 ± 0.13

correlated with direct counts (Table 2).

Treatment of the fibrin clot inside chambers. Chambers were removed from mice after 48 hr of *in vivo* incubation and added to a flask containing 10 ml of HBSS + 5% Pronase (Sigma) which was then rotated at 150 rpm for 55 min at 22-25°C. Pronase treatment adequately dissolved the fibrin clot which forms on the inside of chambers; the number of *C. albicans* cfu recovered after Pronase treatment was similar to the number recovered after homogenization.

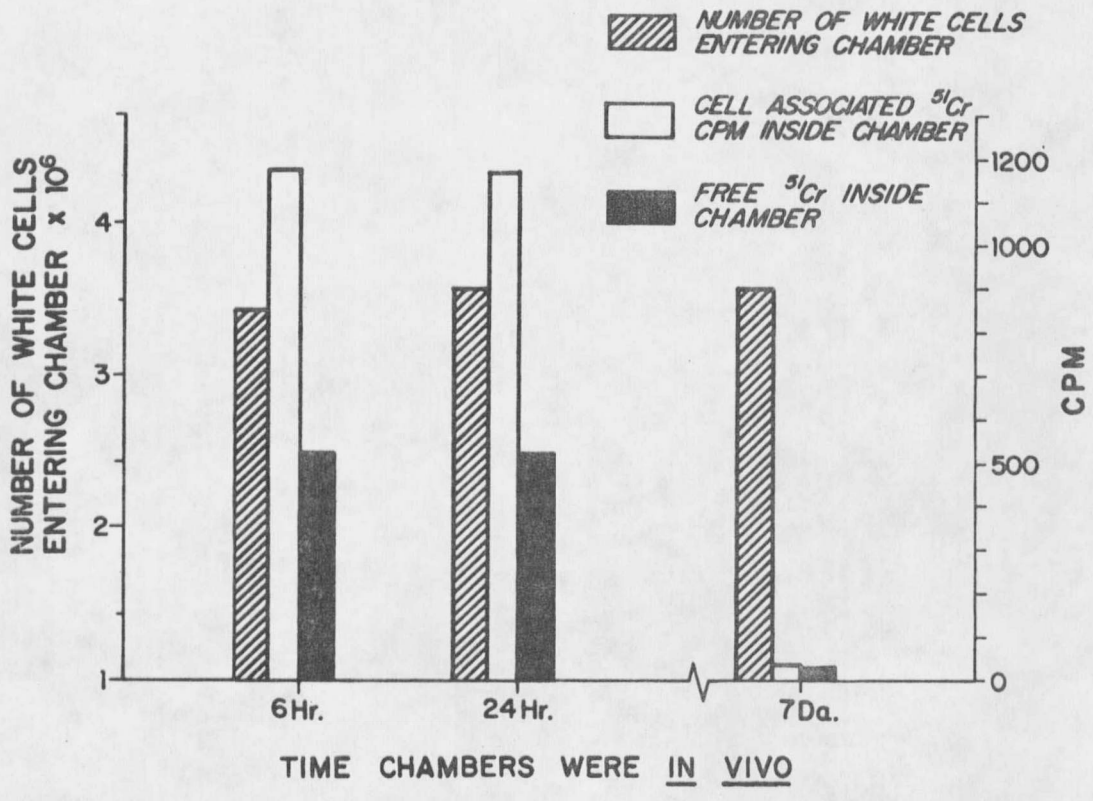
Emigration of host peritoneal cells into 3p chambers. 3p chambers containing yeasts were harvested 6 hr, 24 hr, or 7 days after implantation. The chamber contents were obtained by washing with saline and the number of white cells that entered the chamber was determined by counting in a hemacytometer. About 3.2×10^6 peritoneal cells entered the chambers by 6 hr and their numbers remained constant for 7 days (Figure 2). Microscopic examination of Giemsa stained smears of contents of these chambers showed that in chambers harvested at 6 hr 87% of the cells were neutrophils, and in the chambers harvested at 24 hr 92% of the cells were neutrophils.

In other experiments, ^{51}Cr-labeled neutrophils, ^{51}Cr-labeled spleen cells, or free ^{51}Cr in PBS were injected iv into mice which were implanted with 3p chambers 6 hr, 24 hr, or 7 days previously.

Table 2. Effect of homogenizing filters and chamber contents on the viability of C. albicans in diffusion chambers

Method of recovery of yeasts		Log_{10} no. cfu C. albicans recovered
vortexed -	direct count	7.51 ± 0.05
	plate count	7.55 ± 0.11
homogenized -	plate count	7.62 ± 0.23

Figure 2. Emigration of host peritoneal cells into 3p diffusion chambers


NUMBER OF WHITE CELLS ENTERING CHAMBER
CELL ASSOCIATED 51Cr CPM INSIDE CHAMBER
FREE 51Cr INSIDE CHAMBER
NUMBER OF WHITE CELLS ENTERING CHAMBER x 10^6
4
3
2
1
1200
1000
500
0
CPM
6Hr.
24Hr.
7Da.
TIME CHAMBERS WERE IN VIVO

Six hours after injection, the chamber contents were assayed for cell associated and free ^{51}Cr. Labeled neutrophils and free label entered chambers which were implanted 6 hr or 24 hr before injection of ^{51}Cr, but did not enter chambers which were implanted 7 days earlier (Figure 2). The ^{51}Cr cpm found in chambers when labeled spleen cells were injected was approximately the same as the cpm found when free ^{51}Cr was injected.

Two *in vitro* tests were performed to determine the effect of ^{51}Cr labeling on neutrophil function. In the chemotaxis under agarose test, random migration of ^{51}Cr-labeled neutrophils was 100% of that obtained using unlabeled neutrophils, and in the phagocytosis assay, percent phagocytosis by labeled neutrophils was 100% of that obtained using control neutrophils.

Entry of opsonic factors into diffusion chambers. The ability of opsonic factors to enter diffusion chambers was tested using the *in vitro* phagocytosis assay. Previous work in this and other laboratories (44,34,37) determined that optimum phagocytosis of *C. albicans* by macrophages and neutrophils *in vitro* requires heat labile complement factors present in normal mouse serum. Furthermore, INMS is required for normal macrophage function *in vitro*, but supports only very low levels (<5%) of phagocytosis of *C. albicans* (44). Yeasts taken from chambers after 24 hr of *in vivo* incubation supported a

higher level of phagocytosis when added with INMS than C. albicans that were taken from a culture and were therefore without opsonic factors (Table 3). This indicates that fungal particles contained in the diffusion chambers had been opsonized while in the mouse peritoneal cavity.

Effect of neutrophil-rich PEC on C. albicans in diffusion chambers. Various concentrations of neutrophil-rich PEC were harvested from normal mice, suspended in PBS or NMS, and added with 5×10^4 C. albicans to 0.45p chambers. The chambers were implanted in normal mice and harvested after 24 hr. Regardless of the original suspending medium, neutrophils produced a significant decrease in the number of viable C. albicans when compared to the inoculum at neutrophil to yeast ratios of 400:1 and 40:1 (Table 4).

In other experiments, neutrophil-rich PEC were harvested from nude mice, suspended in PBS and added to 0.45p chambers with 4×10^4 C. albicans. The chambers were implanted in nude mice and harvested after 24 hr of in vivo incubation. A candidacidal effect of nude neutrophils also was seen at 400:1 and 40:1 neutrophil to yeast ratios (Table 5).

To determine if PEC were capable of phagocytizing fungal cells in diffusion chambers, chambers containing 5×10^6 neutrophil-rich PEC and 5×10^4 yeasts were harvested 4 hr after implantation in BALB/c mice.

Table 3. *In vivo* opsonization of *C. albicans* in diffusion chambers

Source of yeasts	Serum[b]	Macrophages % phagocytosis	Neutrophils % phagocytosis
GYEP culture (without opsonic factors	NMS	78.2	88.0
	INMS	8.5	52.0
Diffusion chambers[a]	NMS	64.1 ± 3.1	ND
	INMS	21.8 ± 6.3	81.6 ± 11.8

[a] 0.45 chambers harvested after 24 hr *in vivo* incubation.

[b] Serum added to phagocytosis assay; normal mouse serum (NMS) or NMS absorbed 3 times with *C. albicans* at 37°C (INMS).

Table 4. Effect of neutrophils harvested from normal mice on *C. albicans* in diffusion chambers

No. Neutrophils	N:Y[a] ratio	Log_{10} no. cfu recovered after 24 hrs Neutrophil suspending medium	
		PBS	NMS
$2x10^7$	400:1	3.82 ± 0.19	3.73 ± 0.41
$2x10^6$	40:1	2.68 ± 0.65	3.51 ± 0.77
$2x10^5$	4:1	4.43 ± 0.69	4.87 ± 0.48
$2x10^4$	<1:1	4.87 ± 0.50	5.43 ± 0.26
none	---	5.47 ± 0.44	5.45 ± 0.25

[a] Neutrophil : Yeast ratio; log_{10} 4.69 yeasts were added to chambers.

Table 5. Effect of neutrophils harvested from nude mice on C. albicans in diffusion chambers

No. neutrophils	N:Y[a] ratio	Log_{10} no. cfu recovered after 24 hrs.
$1x10^7$	400:1	3.69 ± 0.24
$1x10^6$	40:1	4.37 ± 0.52
$1x10^5$	4:1	5.02 ± 0.28
$1x10^4$	<1:1	5.75 ± 0.15
none	---	5.25 ± 0.06

[a] Neutrophil : Yeast ratio; log_{10} 4.69 yeasts were added to chambers.

Microscopic examination of Giemsa stained smears of contents of these chambers revealed that 95% of the yeasts in the chambers were phagocytized. Similar levels of phagocytosis were seen when chamber contents containing macrophage-rich PEC were examined.

Effect of macrophage-rich PEC on C. albicans in diffusion chambers. When various concentrations of macrophage-rich PEC harvested from normal mice were suspended in PBS and added to 0.45p chambers with 5×10^4 yeasts, the number of cfu recovered after 24 hr of in vivo incubation was similar to the inoculum, regardless of the number of macrophages added (Table 6). When compared to the number of fungal units recovered from chambers which did not contain macrophages, these data indicate that macrophages harvested from normal mice prevent the growth of C. albicans, but killing of the fungus was not apparent.

The ficoll gradient centrifugation technique was used to further enrich macrophage-rich PEC. The cells obtained by this method were found to be 96% macrophages by examination of Giemsa stained smears. In the in vitro phagocytosis assay, percent phagocytosis by these macrophages was 100% of that obtained using control marcophages. When various concentrations of ficoll gradient enriched macrophages were added to 0.45p chambers with 5×10^4 C. albicans, the result was a candidastatic effect similar to that seen with macrophage-rich PEC that were not further enriched for macrophages (Table 6).

Table 6. Effect of macrophages from normal mice on C. albicans in diffusion chambers

No. macrophages	M:Y[a] ratio	Log_{10} no. cfu recovered after 24 hrs	
		Macrophage-rich PEC	Ficoll gradient purified macrophages
$2x10^7$	400:1	4.66 ± 0.10	ND
$2x10^6$	40:1	4.67 ± 0.18	4.81 ± 0.03
$2x10^5$	4:1	4.89 ± 0.39	4.69 ± 0.68
$2x10^4$	<1:1	4.84 ± 0.38	5.81 ± 0.47
none	---	5.59 ± 0.16	5.92 ± 0.30

[a] Macrophage : Yeast ratio; log_{10} 4.69 yeasts were added to chambers.

When chambers containing macrophage-rich PEC harvested from nude mice and 5×10^4 yeasts were implanted in nude mice and harvested after 24 hr, the number of C. albicans cfu recovered decreased by about 1 log at macrophage to yeast ratios of 400:1 and 40:1 (Table 7). At lower macrophage to yeast ratios a candidastatic effect was seen. To determine if a soluble factor in the peritoneal cavity of the nude mouse was required for the candidacidal activity of nude macrophages, macrophage-rich PEC from nude mice were added to chambers with 5×10^4 C. albicans, and the chambers were implanted in normal mice. In this case, a candidacidal effect occurred at macrophage to yeast ratios of 400:1 and 40:1. Levels of killing were comparable to that seen when nude macrophages were tested in nude mice. Conversely, macrophage-rich PEC harvested from normal mice were added to chambers with 5×10^4 C. albicans, and the chambers were implanted in nude mice. Killing of C. albicans was not seen by these macrophages, but inhibition of growth occurred when compared to control chambers that did not contain macrophages (Table 7).

Table 7. Effect of macrophages harvested from nude mice on *C. albicans* in diffusion chambers

No. macrophages	M:Y[a] ratio	Log_{10} no. cfu recovered after 24 hrs		
		macs from nude→nude	macs from normal→nude	macs from nude→normal
$2x10^7$	400:1	3.94 ± 0.06	4.53 ± 0.08	3.96 ± 0.19
$2x10^6$	40:1	3.77 ± 0.23	4.51 ± 0.13	3.64 ± 0.13
$2x10^5$	4:1	4.59 ± 0.38	4.57 ± 0.23	4.19 ± 0.15
$2x10^4$	<1:1	4.78 ± 0.64	5.05 ± 0.55	4.37 ± 0.38
none	---	5.16 ± 0.71	5.46 ± 0.51	4.76 ± 0.20

[a] Macrophage : Yeast ratio; log_{10} 4.69 yeasts were added to chambers.

DISCUSSION

Immunity to fungal diseases is a complicated issue: T-cell mediated immunity, soluble serum factors, and innate immunity have all been implicated in defense against fungal infection. As part of the innate immunity, phagocytic cells represent a first line of defense against disease. Fungal infection in a normal host usually elicits first a pyogenic response involving primarily neutrophils, followed by a granulomatous response involving macrophages, giant cell formation and lymphocytes. *In vitro* tests by various investigators have led to the assumption that neutrophils and macrophages are capable of killing fungi, such as *Candida albicans* and *Cryptococcus neoformans*, *in vivo*. It was the purpose of this thesis to test this assumption.

Diffusion chambers containing *Candida albicans* implanted in the peritoneal cavity of mice were used to study the effect of host cells and soluble factors on the growth of *C. albicans*. Because this *in vivo* model had not been used in this manner previously, experiments were designed to determine the validity and limitations of this model.

To determine if this model represents a valid method of studying interactions of host factors with *C. albicans*, and to determine limitations of this model, various alterations of experimental methods were made. In some experiments chambers were constructed to allow for exchange of soluble factors only, while in other experiments chambers

were used that should allow for emigration of phagocytic cells as well as diffusion of soluble factors. When host cells were excluded from the chambers, growth of the fungus occurred but the amount of growth was several orders of magnitude lower than the amount of growth seen in chambers *in vitro*. Nonspecific factors such as low oxygen tension or nutrient deprivation may be responsible for the decreased growth seen *in vivo*. Although oxygen tension present in the mouse peritoneal cavity was not determined, if the oxygen tension was low, slow growth of *C. albicans* would be expected (62). It was also found that a fibrin clot formed on the inside of the chambers after about 3 days of *in vivo* incubation, and adhesions between the chamber and organs in the peritoneal cavity formed after about 5 days of *in vivo* incubation. It is reasonable that these contributed to decreased growth *in vivo* by limiting exchange of oxygen and nutrients.

Soluble factors specific for inhibition of growth of *C. albicans* may also be involved in lower *in vivo* growth. Such factors have not been defined by work presented in this thesis, but other workers (39) have noted a factor present in human sera which specifically clumps *C. albicans*. Although it has been suggested that this clumping factor is antibody specific for *Candida*, the presence of clumping factor does not necessarily correlate with the presence of *Candida* agglutinins and precipitins (30). Other workers (38) demonstrated that *Trichophyton mentagrophytes* is inhibited from proliferation in diffusion chambers

implanted in the peritoneal cavity of mice. These authors postulated that a specific soluble factor is present which interferes with the growth of dermatophytes.

The ability of host soluble factors to enter the appropriate chambers was determined in two ways. An in vitro phagocytosis assay was used to test for the presence of opsonic factors on yeast particles in diffusion chambers. It was found that opsonins, most likely complement factors (44), were able to enter chambers that were in the peritoneal cavity for 24 hr. In experiments in which free ^{51}Cr was injected iv into mice shortly after implantation of diffusion chambers, the radionuclide was detectable in chambers implanted 24 hr before injection but would not enter chambers that were in the peritoneal cavity for 7 days prior to injection. This lack of exchange of fluids may be due to the fibrin clot formation and adhesions.

The ability of cells to enter chambers implanted ip was determined by injecting ^{51}Cr-labeled neutrophils into mice containing chambers. These cells were found able to enter chambers which had been implanted 24 hr earlier, but could not enter chambers which had been in the peritoneal cavity for 7 days. The inability of cells to emigrate into these 7 day-chambers correlates with the inability of soluble factors (free ^{51}Cr) to enter similar chambers.

Various tests indicated that the ^{51}Cr cpm detected in 24 hr-

chambers correlated with the entry of phagocytic cells into the chambers. The ^{51}Cr cpm that was found in chambers when labeled spleen cells were injected into mice could be due to the emigration into chambers by phagocytic cells which are normally resident in the spleen, or due to the lysis of spleen cells which would result in release of ^{51}Cr. Seventy percent of ^{51}Cr cpm found in chambers when labeled neutrophils were injected was cell associated and labeled spleen cells did not enter chambers to the extent of labeled neutrophils. Also, labeled neutrophils were found to be functionally active by the *in vitro* phagocytosis and random migration assays. Collectively these data suggest that active phagocytic cell emigration into chambers was occurring.

From these various experiments, it appears that the diffusion chamber *in vivo* is a system where conditions change over a period of time. Soluble factors and phagocytic cells can enter chambers in the mouse peritoneal cavity for at least 24 hr. In chambers which have been in the peritoneal cavity for 7 days, the internal fibrin clot and external adhesions are evident and may prevent soluble factors and cells from entering chambers.

With this knowledge of the diffusion chamber system, a series of experiments was designed to study the effects of various host components on the growth of *C. albicans* in diffusion chambers. Experiments

were first performed to examine the effects of allowing natural emigration of host factors (cells and soluble components) into chambers containing C. albicans. The fate of C. albicans under such conditions was dependent on the concentration of yeasts placed in the chamber. Host peritoneal cells were found to be significantly candidacidal at day 1 only when the smaller inoculum of C. albicans was used. An increase in viable C. albicans occurred after day 1 -- by day 7 the number of yeasts was similar to the number in the original inoculum. The growth of C. albicans after day 1 may be due to the inability of host cells to enter chambers. Stasis or a slight decrease in viability was seen at the higher yeast inoculum. In other experiments, it was determined that just over 10^6 white cells entered chambers. It is possible that this number of white cells was insufficient for effective candidacidal activity when the higher inoculum of yeasts was used.

The decrease in viability of C. albicans observed at day 1 was not due to mechanical damage incurred by C. albicans during the harvest-homogenization procedure. This possibility was ruled out by placing chambers containing C. albicans in vitro and comparing the number of viable fungal units recovered from chambers by homogenizing to the number recovered by simple vortexing. Similar numbers of C. albicans were recovered by both methods. Thus it appears that a decrease in

viable C. albicans in chambers incubated in vivo for 24 hr is due to host candidacidal mechanisms.

Examination of Giemsa stained smears of contents of chambers which were in vivo for 24 hr showed the presence of neutrophils and some macrophages. Therefore, to determine the effect of each of these cell types on C. albicans, neutrophil-rich PEC from normal mice were added with C. albicans to diffusion chambers designed to allow for exchange of soluble substances in the mouse peritoneal cavity. Under these conditions, neutrophils were found to be candidacidal, but only at high neutrophil to yeast ratios. By 4 hr incubation in chambers, it was found that nearly all of the fungal particles were ingested by neutrophils and, surprisingly, even at low neutrophil to yeast ratios. The high neutrophil to yeast ratio requirement for candidacidal activity of neutrophils correlates with previous experiments using C. albicans in chambers that allow emigration of host peritoneal cells: a candidacidal effect was seen only in chambers containing the smaller yeast inoculum. This evidence suggests that ingestion of a yeast by a neutrophil merely "weakens" the yeast, but a second or third neutrophil is required for killing.

The idea that ingestion of C. albicans by a neutrophil does not necessarily lead to death of the fungus has also been proposed by others (68). These workers observed that C. albicans was able to

persist *in vivo* by germinating out of phagocytic cells. Other workers have shown that neutrophils can affect *Candida* cells after germination of the yeasts. Diamond and coworkers have demonstrated the existance of an interaction between *Candida* and neutrophils *in vitro* under conditions in which the *Candida* cells are not ingested (17,18). They showed that neutrophils could attach and spread out over *Candida* pseudohyphae; this was followed by degraulation of neutrophils with morphologic and metabolic changes in the pseudohyphae suggestive of severe cell damage or death. A similar interaction was shown between neutrophils and *Aspergillus* and *Mucor* (16).

Other experiments were performed to determine if addition of normal mouse serum would enhance the candidacidal effect of neutrophils in chambers. Results of these experiments indicated that serum did not affect the number of viable *C. albicans* recovered. This was not a surprising result since other workers have shown that serum is necessary for optimal phagocytosis of *Candida* by neutrophils *in vitro* but serum does not contribute to the candidacidal activity (34,37). My experiments suggest that fluid factors in the peritoneal cavity provide sufficient opsonins: phagocytosis of *Candida* by neutrophils in chambers was shown to be nearly total, and yeasts taken from chambers did not require normal mouse serum for optimal phagocytosis by neutrophils and macrophages *in vitro*.

Since macrophages are also seen at foci of Candida infections, the effect of macrophages on C. albicans in diffusion chambers was examined. Macrophage-rich PEC harvested from normal mice and added to chambers with yeasts were found to have only candidastatic effects at both high and low macrophage to yeast ratios. The possibility that this candidastatic effect was due to neutrophils in the macrophage population was addressed by further enriching the cell population for macrophages by ficoll gradient centrifugation. The cell population obtained through this technique was over 96% macrophages; when these cells were added to chambers with yeasts, a similar candidastatic effect was seen. Thus inhibition of fungal growth does appear to be due to macrophage activity.

Other workers have examined the candidacidal effects of macrophages and neutrophils in vitro. The comparative candidacidal activities of these two cell types is a controversial subject. Leijh et al (37) found that neutrophils killed 58% of ingested yeasts while monocytes killed only 50%, whereas Lehrer and Cline (34) found that neutrophils killed 29% of ingested C. albicans while monocytes killed only 4%. Conversely, Schuit (59) found monocytes to be more candidacidal: monocytes were able to kill 60% of ingested C. albicans blastospores and 50% of ingested psuedohyphae while neutrophils killed only 35% of ingested blastospores and about 5% of ingested pseudohyphae.

In yet other studies, 60% killing of C. albicans ingested by guinea pig neutrophils was reported (4).

It has been postulated that nude mice possess macrophages that are "activated" (12) because these animals are more resistant to the intracellular pathogens Brucella and Listeria. Nude mice have also been found to be more resistant to acute Candida infections (14,54); a result that may be explained by increased macrophage activity. This possibility was addressed by testing the candidacidal activity of macrophages harvested from nude mice in diffusion chambers. In contrast to macrophages from normal mice which were candidastatic, macrophages from nude mice were found to be candidacidal at high macrophage to yeast ratios when added with yeasts to chambers and implanted in either nude or normal mice. If this candidacidal effect was due to an interaction of macrophages with a soluble factor present in the nude peritoneal cavity then macrophages from normal mice would be expected to be candidacidal if incubated in nude mice. A change from candidastatic to candidacidal activity of macrophages from normal mice was not observed, therefore demonstrating that nude macrophages alone are candidacidal.

Although nude macrophages are candidacidal in diffusion chambers, one can only speculate about the role of these candidacidal macrophages in the increased resistance to acute candidiasis in nude mice. In one

study attempts were made to determine if "activated" macrophages were involved in defense against candidiasis (55). These workers nonspecifically activated macrophages in BALB/c mice using BCG. Although BCG treatment lessened the severity of Listeria infections, these mice were not more resistant to C. albicans infections.

In conclusion, our results studying the effect of mouse peritoneal exudate cells on Candida albicans in diffusion chambers in vivo agree with in vitro results of other investigators (34,37): neutrophils were found to be candidacidal and macrophages were less candidacidal but did inhibit growth of C. albicans. Also, this work indicates that macrophages from nude mice were candidacidal. Diffusion chambers implanted in the peritoneal cavity of mice can be used to study the effect of specific cell populations on fungi in vivo. Further studies might include use of sensitized animals, sensitized lymphocytes added to chambers, and use of mixtures of different cell populations to examine host modulation of cellular and soluble factor effects on fungi.

LITERATURE CITED

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1. Amos, D.B., and J.D. Wakefield. 1958. Growth of mouse ascites tumor cells in diffusion chambers. I. Studies of growth rates of cells and of entry of antibody. J. Nat. Canc. Inst. 21:657-670.

2. Amos, D.B. and J.D. Wakefield. 1959. Growth of mouse ascites tumor cells in diffusion chambers. II. Lysis and growth inhibition by diffusible isoantibody. J. Nat. Canc. Inst. 22:1077-1092.

3. Amos, D.B. 1961. Transplantation of cells and tissues in diffusion chambers. In Transplantation of Tissues and Cells Ed. by R.E. Billingham. Wistar Inst. Press. Philadelphia.

4. Arai, T., Y. Mikami, and K. Yokoyama. 1977. Phagocytosis of Candida albicans by rabbit alveolar macrophages and guinea pig neutrophils. Sabouraudia 15:171-177.

5. Benestad, H.B. 1970. Formation of granulocytes and macrophages in diffusion chamber cultures of mouse blood leukocytes. Scand. J. Haemat. 7:279-288.

6. Benestad, H.B. and A. Reikvam. 1975. Diffusion chamber culturing of haematopoietic cells: methodological investigations and improvement of the technique. Exp. Hematol. 3:249-260.

7. Blyth, W. and G.E. Stewart. 1978. Systemic candidiasis in mice treated with prednisolone and amphotericin B. I. Morbidity, mortality, and inflammatory reaction. Mycopathologia 66:41-50.

8. Boyum, A., W. Boecker, A.L. Carsten, and E.P. Cronkite. 1972. Proliferation of human bone marrow cells in diffusion chambers implanted into normal or irradiated mice. Blood 40:163.

9. Boyum, A., A.L. Carsten, O.D. Laerum, and E.P. Cronkite. 1972. Kinetics of cell proliferation of murine bone marrow cells cultured in diffusion chambers: effect of hypoxia, bleeding, erythropoietin injections, polyeythemia, and irradiation of the host. Blood 40:174.

10. Breivik, H., H.B. Benestad, and A. Boyum. 1971. Diffusion chamber and spleen colony assay of murine haematopoietic stem cells. J. Cell. Physiol. 78:65-72.

11. Breivik, H. 1971. Haematopoietic stem cell content of murine bone marrow, spleen, and blood. Limiting dilution analysis of diffusion chamber cultures. J. Cell. Physiol. 78:73-78.

12. Cheers, C. and R. Waller. 1975. Activated macrophages in congenitally athymic "nude" mice and in lethally irradiated mice. J. Immunol. 115:844-847.

13. Corbel, M.J. and S.M. Eades. 1976. The relative susceptibility of New Zealand Black and CBA mice to infection with opportunistic fungal pathogens. Sabouraudia 14:17-32.

14. Cutler, J.E. 1976. Acute systemic candidiasis in normal and congenitally athymic-deficient (nude) mice. J. Reticuloendothel. Soc. 19:121-124.

15. Cutler, J.E. 1977. Chemotactic factor produced by Candida albicans. Infec. Immun. 18:568-573.

16. Diamond, R.D., R. Krzesicki, B. Epstein, and W. Jao. 1978. Damage to hyphal forms of fungi by human leukocytes in vitro. Amer. J. Pathol. 91:313-328.

17. Diamond, R.D., R. Krzesicki and W. Jao. 1978. Damage to pseudohyphal forms of Candida albicans by neutrophils in the absence of serum in vitro. J. Clin. Invest. 61:349-359.

18. Diamond, R.D. and R. Krzesicki. 1978. Mechanisms of attachment of neutrophils to Candida albicans pseudohyphae in the absence of serum, and of subsequent damage to pseudohyphae by microbicidal processes of neutrophils in vitro. J. Clin. Invest. 61:360-369.

19. Djawari, D., O.P. Hornstein, J. Gross, and W. Meinhof. 1977. Defect of phagocytosis and intracellular killing of Candida albicans by granulocytes in patients with familiar and non-familiar chronic mucocutaneous candidosis. Arch. Dermtol. Res. Lett. 260:159-161.

20. Domer, J.A., and S.A. Moser. 1978. Experimental murine candidosis: cell-mediated immunity after cutaneous challenge. Infect. Immun. 20:88-98.

21. Edwards, J.E., R.I. Lehrer, E.R. Stiehm, T.H. Fischer, and L.S. Young. 1978. Severe candidal infections. Clinical perspective, immune defense mechanisms, and current concepts of therapy. Ann. Int. Med. 89:91-106.

22. Elin, R.J., J.B. Edelin, and S.M. Wolff. 1974. Infection and immunoglobulin concentrations in Chediak-Higashi mice. Infec. Immun. 10:88-91.

23. Emmons, C.W., C.H. Binford, J.P. Utz, and K.J. Kwon-Chung. 1977. Medical Mycology. Lea and Febiger. Philadelphia.

24. Fauerholdt, L. and N. Jacobsen. 1975. Cultivation of leukemic human bone marrow cells in diffusion chambers implanted into normal and irradiated mice. Blood 45:495-501.

25. Ford, W.L. and S.V. Hunt. 1973. The preparation and labelling of lymphocytes. In Applications of Immunological Methods. Ed. by D.M. Weir. F.A. Davis, Philadelphia.

26. Gallin , J.E., J.S. Bujak, E. Patten, and S.M. Wolff. 1974. Granulocyte function in the Chediak-Higashi syndrome of mice. Blood 43:201-206.

27. Gelfand, J.A., D.L. Hurley, A.S. Fauci, and M.M. Frank. 1978. Role of complement in host defense against experimental disseminated candidiasis. J. Infec. Dis. 138:9-16.

28. Giger, D.K., J.E. Domer, and J.T. McQuitty. 1978. Experimental murine candidiasis: pathological and immune responses to cutaneous inoculation with Candida albicans. Infec. Immun. 19:499-509.

29. Giger, D.K., J.E. Domer, S.A. Moser, and J.T. McQuitty. 1978. Experimental murine candidiasis: pathological and immune response in T-lymphocyte-depleted mice. Infec. Immun. 21:729-737.

30. Hasenclever, H.F. 1978. The in vitro interactions of Candida albicans with nonspecific serum proteins. Mycopathologia 65:169-176.

31. Jacobsen, N. and L. Fauerholdt. 1976. Human granulocytopoietic colonies in diffusion chambers in mice: growth of colonies and the effect of host irradiation. Scand. J. Haematol. 16:101-111.

32. Johnson, J.A., B.H.S. Lau, R.L. Nutter, J.M. Slater, and C.E. Winter. 1978. Effect of L1210 leukemia on the susceptibility of mice to Candida albicans infections. Infec. Immun. 19: 146-151.

33. Kirkpatrick, C.H., R.R. Rich, and J.E. Bennett. 1971. Chronic mucocutaneous candidiasis: model-building in cellular immunity. Ann. Int. Med. 74:955-978.

34. Lehrer, R.I., and M.J. Cline. 1969. Interaction of Candida albicans with human leukocytes and serum. J. Bacteriol. 98: 996-1004.

35. Lehrer, R.I. and M.J. Cline. 1969. Leukocyte myeloperoxidase deficiency and disseminated candidiasis: the role of myeloperoxidase in resistance to Candida infection. J. Clin. Invest. 48:1478-1488.

36. Lehrer, R.I. 1970. Measurement of candidacidal activity of specific leukocyte types in a mixed cell population. I. Normal, myeloperoxidase deficient, and chronic granulomatous disease neutrophils. Infec. Immun. 2:42-47.

37. Leijh, P.C.J., M.T. van den Barselaar, and R. van Furth. 1977. Kinetics and phagocytosis and intracellular killing of Candida albicans by human granulocytes and monocytes. Infec. Immun. 17:313-318.

38. Lorincz, A.L., J.O. Priestley, and J.H. Jacobs. 1958. Evidence for a humoral mechanism which prevents growth of dermatophytes. J. Invest. Dermatol. 31:15-17.

39. Louria, D.B., J.K. Smith, R.G. Brayton and M. Buse. 1972. Anti-Candida factors in serum and their inhibitors. I. Clinical and Laboratory Observations. J. Infec. Dis. 125: 102-114.

40. Mason, B.H., and B.F. Cain. 1978. Attempted drug-tumor sensitivity matching using diffusion chamber-cultivated cells. Canc. Trtmt. Rep. 62:2035-2043.

41. McPherson, C.W. 1963. Reduction of Pseudomonas aeruginosa and coliform bacteria in mouse drinking water following treatment hydrochloric acid or chlorine. Lab. Anim. Care 13:737-744.

42. Mishell, R.I. and R.W. Dutton. 1967. Immunization of dissociated spleen cell cultures from normal mice. J. Exp. Med. 126: 423-454.

43. Miyake, T., K. Takeya, K. Nomoto, and S. Muraka. 1977. Cellular elements in the resistance to Candida infection in mice. I. Contribution of T lymphocytes and phagocytes at various stages of infection. Microbiol. Immunol. 21:703-725.

44. Morrison, R.P. and J.E. Cutler. Serum factors required for phagocytosis of Candida albicans by mouse macrophages in vitro. Manuscript in preparation.

45. Myerowitz, R.L., G.J. Pazin, and C.M. Allen. 1977. Disseminated candidiasis. Changes in incidence, underlying diseases, and pathology. Amer. J. Clin. Path. 68:29-38.

46. Nettesheim, P., T. Makinodan and C.J. Chadwick. 1966. Improved diffusion chamber cultures for cytokinetic analysis of antibody response. Immunology 11:427-439.

47. Oh, M.K., G.E. Rodey, R.A. Good, R.A. Chilgren, and P.G. Quie. 1969. Defective candidacidal capacity of polymorphonuclear leukocytes in chronic granulomatous disease of childhood. J. Pediat. 75:300-303.

48. Parker, J.C., J.J. McCloskey, and R.S. Lee. 1978. Emergence of candidosis. The dominant postmortem cerebral mycosis. Amer. J. Clin. Path. 70:31-36.

49. Pearsall, N.N. and D. Lagunoff. 1974. Immunological responses to Candida albicans. I. Mouse-thigh lesion as a model of experimental candidiasis. Infec. Immun. 9:999-1002.

50. Pilgrim and DeOme. 1955. Intraperitoneal pentobarbital anesthesia in mice. Exp. Med. Surg. 13:401-403.

51. Quie, P.G., and R.A. Chilgren. 1971. Acute disseminated and chronic mucocutaneous candidiasis. Sem. Hematol. 8:227-242.

52. Ray, T.L., and K.D. Wuepper. 1976. Activation of the alternative (properdin) pathway of complement by Candida albicans and related species. J. Invest. Dermatol. 67:700-703.

53. Ritz, H. 1965. Isolation of complement fractions presents a new approach to immunosuppression. J. Amer. Med. Assoc. 191:30-31.

54. Rogers, T.J., E. Balish, and D.D. Manning. 1976. The role of thymus-dependent cell-mediated immunity in resistance to experimental disseminated candidiasis. J. Reticuloendothel. Soc. 20:291-298.

55. Rogers. T.J. and E. Balish. 1977. The role of activated macrophages in resistance to experimental renal candidiasis. J. Reticuloendothel. Soc. 22:309-318.

56. Rohrer, J.W., K. Vasa, and R.G. Lynch. 1977. Myeloma cell immunoglobulin expression during in vivo growth in diffusion chambers: evidence for repetitive cycles of differentiation. J. Immunol. 119:861-866.

57. Rohrer, J.W. and R.G. Lynch. 1977. Specific, immunoglobulin regulation of differentiation of immunoglobulin expression in MOPC-315 cells during in vivo growth in diffusion chambers. J. Immunol. 119:2045-2053.

58. Schmid, L., and K. Brune. 1974. Assessment of phagocytic and antimicrobial activity of human granulocytes. Infec. Immun. 10:1120-1126.

59. Schuit, K.E. 1979. Phagocytosis and killing of pathogenic yeasts by human monocytes and neutrophils. Infec. Immun. 24:932-938.

60. Snyderman, R., L.C. Altman, A. Frankel, and R.M. Blaese. 1973. Defective mononuclear leukocyte chemotaxis: a previously unrecognized immune dysfunction. Studies in a patient with chronic mucocutaneous candidiasis. Ann. Int. Med. 78:509-513.

61. Staples, P.J., J. Boujak, R.G. Douglas, and J.P. Leddy. 1977. Disseminated candidiasis in a previously healthy girl: implication of a leukocyte candidacidal defect. Clin. Immunol. Immunopath. 7:157-167.

62. Szawatkowski, M. and J.M.T. Hamilton-Miller. 1979. Anaerobic growth and sensitivity of Candida albicans. Microbios. Lett. 5:61-66.

63. Tyler, W.S., F. Stohlman, J. Keane, M. Chovaneic, and D. Howard. 1974. Survival of mouse hemopoietic cells in an in vivo culture system. Proc. Soc. Exp. Biol. Med. 146:316-320.

64. Valdimarsson, H., J.M. Higgs, R.S. Wells, M. Yamamura, J.R. Hobbs, and P.J.L. Holt. 1973. Immune abnormalities associated with chronic mucocutaneous candidiasis. Cell. Immunol. 6:348-361.

65. Warden, G.K., A.D. Mason, and B.A. Pruitt. 1974. Evaluation of leukocyte chemotaxis in vitro in thermally injured patients. J. Clin. Invest. 54:1001-1004.

66. White, B.E. 1972. Cerebral candidiasis. N. Engl. J. Med. 286: 321.

67. Wolff, S.M., D.C. Dale, R.A. Clark, R.K. Root, and H.R. Kimball. 1972. The Chadiak-Higashi syndrome: studies of host defenses. Ann. Int. Med. 76:293-306.

68. Young, G. 1958. The process of invasion and the persistence of Candida albicans injected intraperitoneally into mice. J. Infec. Dis. 102:114-120.

69. Zembala, M. and G.L. Asherson. 1970. The rapid purification of peritoneal exudate macrophages by ficoll (polysucrose) density gradient centrifugation. Immunology 19:677-681.

APPENDICES

APPENDIX I

Effect of neutrophils on Streptococcus pneumoniae in diffusion chambers

Streptococcus pneumoniae is a pyogenic coccus which is pathogenic for man because of its invasive properties. This organism possesses an antiphagocytic capsule; anti-capsular antibody acts as an opsonin thus allowing phagocytic cells to ingest the bacteria. These bacteria are readily killed once ingested.

The goal of these experiments was to demonstrate the ability of antibody to diffuse into the chambers and specifically combine with S. pneumoniae, and to demonstrate the ability of neutrophils to phagocytize and kill opsonized bacteria. Neutrophils and S. pneumoniae were added to chambers which were implanted in S. pneumoniae-immunized and nonimmunized mice. We would expect neutrophil bacterialcidal activity only in chambers implanted in immunized mice.

S. pneumoniae, type III, obtained from the culture collection at Montana State University, was grown on brain heart infusion (BHI) agar containing 5% sheep erythrocytes for 18 hr at 37°C in a candle jar. An isolated colony from this plate was inoculated into BHI broth containing 15% FCS and incubated in a candle jar for 12 hr at 37°C. The bacteria were harvested by centrifugation at 10,000 x g for 30 min, suspended in PBS, and the suspension was lyophilized. This material was suspended in PBS to the proper concentration and used as the antigen for immunization. Others have determined that the optimal immuniz-

ing dose for purified S. pneumoniae SSS III capsular polysaccharide is 0.5 µg (3). Assuming that the capsule represents 15% of the dry weight of S. pneumoniae cells, mice were immunized with 1.67 µg of the antigen preparation. Eight to twelve week old male or female BALB/c mice were immunized ip on days 0, 7, and 28. On day 35, S. pneumoniae, cultured as described above, was suspended to the appropriate concentration in PBS using McFarland's standards as a reference. The bacteria were added to 0.45p chambers with or without neutrophil-rich PEC harvested from normal mice. The chambers were implanted ip into immunized or nonimmunized normal mice and harvested after 24 hr of in vivo incubation. Cfu of S. pneumoniae remaining in the chambers was determined by homogenizing the filters and chamber contents in 5 ml of saline, plating the homogenates on BHI blood agar, and incubating the plates in a candle jar at 37°C for 12 hr.

In experiment #1, a significant difference in the number of S. pneumoniae recovered from group A chambers was found when compared to the number recovered from groups B, C and D (Table 8). These data support the contention that antibody can enter diffusion chambers and neutrophils in diffusion chambers can ingest and kill opsonized bacteria. However, similar results were not obtained in subsequent experiments. It is possible that the S. pneumoniae inoculum in the second experiment was too high for effective opsonization of bacterial-

Table 8. Effect of neutrophils on Streptococcus pneumoniae in diffusion chambers

Group	Mice immunized[a]	Neutrophils added to chamber[b]	Log_{10} cfu recovered after 24 hrs Exp #1[c]	Exp #2[d]	Exp #3[c]
A	+	+	6.35 ± 0.40	8.3 ± 0.29	7.55 ± 0.06
B	+	-	7.40 ± 0.31	8.8 ± 0.37	7.55 ± 0.16
C	-	+	7.34 ± 0.46	9.76 ± 0.11	7.67 ± 0.13
D	-	-	7.46 ± 0.26	10.1 ± 0.24	7.58 ± 0.16

[a] Mice were immunized ip with 1.67 µg of S. pneumoniae on days 7, 24, and 28 prior to testing.

[b] 10^7 neutrophil-rich PEC were added to chambers.

[c] S. pneumoniae inoculum was about $1x10^6$.

[d] S. pneumoniae inoculum was about $6x10^8$.

cidal activity of neutrophils. Before the third experiment was performed, the stock culture of S. pneumoniae used in previous experiments was lost and a new stock culture was made from previously frozen ($-20^{o}C$) cultures of S. pneumoniae. Although this second stock culture was thought to be of the same capsular type as the original strain, one explanation for the failure of experiment #3 is that the mice were not immunized against the proper capsular type of S. pneumoniae. Also, mice were immunized with the equivalent of 0.25 µg of S. pneumoniae capsular material; doubling the immunizing dose would result in increased antibody titers (3).

APPENDIX II

Effect of silica on the phagocytic ability of mice.

Phagocytic cells, macrophages and neutrophils, are thought to play a role in defense against candidiasis. Treatment of mice with the macrophage toxin silica (7) is a method of *in vivo* depletion. Silica had been used in the past to identify host defense mechanisms to a variety of organisms: silica treated mice are more susceptible to certain viral (11), parasitic (8), and bacterial infections (1,6). The purpose of these experiments was to determine if silica treated mice are more susceptible to infection by *Candida albicans*. Preliminary experiments were performed to determine the effect of silica treatment on the clearance of *C. albicans* and carbon from the blood of normal mice. Diffusion chamber experiments were begun at that point so no further silica experiments were done.

Silica, Min-U-Sil #216 (Whittaker, Clark, and Daniels, Inc. South Plainsfield, NJ) of less than 5 μm diameter was prepared using the method of others (4). Silica prepared in this manner was suspended to the desired concentration in PBS, sonicated for 3 min, and administered iv in 0.25 ml to 8-12 week old male or female BALB/c mice. Eighteen to twenty hours after silica injection, the phagocytic index of normal and silica treated mice was assessed by measuring the ability of the mice to clear *C. albicans* and carbon from the blood.

C. albicans was suspended in PBS to 1 x 10^8 cells/0.1 ml and 0.1 ml was injected iv. Five min (or, in some experiments, 2 min) and 15 min after injection of the yeasts, mice were bled from the orbital venous plexus using a heparinized capillary pipette. At each bleeding 0.05 ml of blood was added to 0.9 ml of PBS and appropriate dilutions were plated onto sabouraud-dextrose agar. Cfu of C. albicans were counted after 36 hr incubation at 37°C.

Carbon clearance was determined using a modification (9) of a method of others (5). Mice were weighed, bled from the orbit and injected iv with 0.01 ml per gram body weight of a 10 mg/ml colloidal carbon (Pelikan ink, Gunther Wagner, Hanover, Germany) in 1% gelatin solution. Two min and 15 min after injection of the carbon, mice were bled from the orbit. At each bleeding 0.05 ml of blood was lysed in 2.0 ml of 0.1% Na_2CO_3. Optical densities (O.D.) were determined at 640 nm (Varian Techtron Model 635 spectrophotometer). The phagocytic index, K, was determined for each mouse by the formula:

$$K = \frac{\log_{10} \text{O.D.}_{\ 2\ \text{min}} - \log_{10} \text{O.D.}_{\ 15\ \text{min}}}{10}$$

The phagocytic index was corrected for differences in body and organ weight, α, by the formula:

$$\alpha = K \times 3 \quad \text{Liver + spleen weight/body weight}$$

Silica treatment is expected to decrease the ability of mice to clear particulate matter from the blood. Clearance of C. albicans from the blood appeared to be more efficient in silica treated mice than in normal mice (Table 9). Since silica has not been noted to enhance macrophage function, carbon clearance assays were performed to determine if the silica treatment was impairing RES function. Macrophage toxicity was found to be silica dose dependent (Table 10). Carbon clearance was slightly decreased if mice were treated with 10 mg of silica, and greatly decreased if mice were treated with 12.5 mg of silica. Doses of silica similar to those given in the Candida clearance assay had no effect on the ability of mice to clear carbon. Low doses of silica (2.5-5 mg) did not seem to adversely affect the health of the mice, but about on-half of the mice treated with the high dose of silica (12.5 mg) died within 12 hr of injection. Thus only very toxic doses of silica were able to depress macrophage function.

Table 9. Clearance of Candida albicans from blood of normal and silica treated BALB/c mice

Silica injected[a]	Log_{10} no. C. albicans[b] cfu recovered from blood	
	5 min.	15 min.
none	5.01 ± 0.24	4.56 ± 0.38
5 mg	4.98 ± 0.44	3.85 ± 0.07
	2 min.	15 min.
none	6.12 ± 0.15	4.94 ± 0.16
2.5 mg	6.01 ± 0.08	4.59 ± 0.20
5 mg	6.23 ± 0.06	4.75 ± 0.12

[a] silica injected iv 18 hrs. previous to Candida clearance test

[b] $1x10^8$ C. albicans injected at time 0

Table 10. Effect of silica on the phagocytic ability of BALB/ mice[a]

Silica dose[b]	Phagocytic index[c]
none	0.117 ± 0.002
4 mg	0.116 ± 0.01
10 mg	0.111 ± 0.013
12.5 mg	0.040 ± 0.003

[a] Phagocytic ability was determined using the carbon clearance assay.

[b] Silica was given i.v. 18-20 hrs. before carbon clearance assay.

[c] Average value ± S.D. of 3-4 mice.

APPENDIX III

Effect of phagocytic cells from normal mice on C. albicans in diffusion chambers in the presence of immune serum.

Other workers (2) have shown that neutrophils and macrophages display increased phagocytosis and increased killing of C. albicans in the presence of anti-C. albicans immune serum. I attempted to determine the effect of immune serum on the candiacidal abilities of mouse neutrophils and macrophages in diffusion chambers.

Anti-C. albicans serum (immune serum) was obtained as previously described (10). Briefly, 4×10^8 heat killed C. albicans were emulsified in complete Freund adjuvant (GIBCO) and injected subcutaneously into rabbits. Booster injections of heat killed cells emulsified in incomplete Freund adjuvant were given on days 7 and 14 and on day 24 the animals were bled. The C. albicans agglutinin titer of sera obtained from these animals was at least 256. This serum was absorbed 3 times with BALB/c spleen cells at $4^{\circ}C$ and was kept at $-20^{\circ}C$ until use. Neutrophil-rich and macrophage-rich PEC were harvested as previously described and suspended to various concentrations in immune serum. The neutrophils or macrophages were added to 0.45p chambers with 5×10^4 C. albicans and the chambers were implanted ip into normal mice and harvested after 24 hr of in vivo incubation.

Neutrophil-rich PEC appear to be less candidacidal when suspended in immune serum than when suspended in PBS (Table 11; Table 4 in

Results section of this thesis). Also, the number of cfu recovered from chambers containing macrophages in immune serum is somewhat higher than the number recovered from chambers containing macrophages in PBS (Table 11: Table 6 in Results section). The effect of immune serum on phagocytic cell candidacidal ability that accounts for these differences can not be determined by these experiments.

Table 11. Effect of phagocytic cells from normal mice on C. albicans in diffusion chambers in the presence of immune serum

No. phagocytic cells	P:Y[a] ratio	Log_{10} No. cfu recovered after 24 hr Phagocytic cell in chamber	
		Neutrophil	Macrophage
2×10^7	400:1	4.29 ± 0.10	4.42 ± 0.10
2×10^6	40:1	3.90 ± 0.39	4.88 ± 0.32
2×10^5	4:1	4.75 ± 0.15	5.13 ± 0.31
2×10^4	<1:1	4.88 ± 0.29	5.44 ± 0.15
none	---	5.42 ± 0.31	5.56 ± 0.10

[a] Phagocyte to yeast ratio; Log_{10} 4.69 yeasts were added to chambers

REFERENCES TO APPENDICES

1. Allison, A.C., and P. D'Arcy Hart. 1968. Potentiation by silica of the growth of Mycobacterium tuberculosis in macrophage cultures. Br. J. Exptl. Path. 49:465.

2. Arai, T., Y. Mikami, and K. Yokoyama. 1977. Phagocytosis of Candida albicans by rabbit alveolar macrophages and guinea pig neutrophils. Sabouraudia 15:171-177.

3. Baker. P.J., P.W. Stashak, D.F. Amsbaugh and B. Prescott. 1971. Characterization of the antibody response to Type III pneumococcal polysaccharide at the cellular level. I. Dose-response studies and the effect of prior immunization on the magnitude of the antibody response. Immunology 20:469-480.

4. Becker, C.J. and J.A. Rudbach. 1979. Altered antibody responses in mice treated with toxins for macrophages. J. Reticuloendoth. Soc. 25:443-454.

5. Biozzi, G., B. Benacerraf, and B.N. Halpern. 1953. Quantitative study of the granulopectic activity of the reticuloendothelial system. II. A study of the kinetics of the granulopectic activity of the RES in relation to the dose of carbon injected: relationship between the weight of the organs and their activity. Br. J. Exp. Pathol. 34:441.

6. Fridman, R.L., and R.J. Moon. 1977. Hepatic clearance of Salmonella typhimurium in silica-treated mice. Infec. Immun. 16:1005.

7. Kessel, R.W.I., L. Monaco, and M.A. Marchisio. 1963. The specificity of the cytotoxic action of silica-a study in vitro. Br. J. Exp. Pathol. 44:351.

8. Kierszenbaum, F., E. Knecht, D.B. Budzko, and M.C. Pizzimenti. 1974. Phagocytosis: a defense mechanism against infection with Trypanosoma cruzi. J. Immunol. 112:1839.

9. Levy, M.H., and E.F. Wheelock. 1975. Effects of intravenous silica on immune and nonimmune functions of the murine host. J. Immunol. 115:41.

10. Poor, A.H. and J.E. Cutler. 1979. Partially purified antibodies used in a solid-phase radioimmunoassay for detecting candidal antigenemia. J. Clin. Microbiol. 9:362-368.

11. Wirth, J.J., M.H. Levy, and E.F. Wheelock. 1976. Use of silica to identify host mechanisms involved in suppression of established Friend virus leukemia. J. Immunol. 117:2124.

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