



Characterization of mouse antibody responses to *Candida albicans*
by Gregory Emiel Winterrowd

A thesis submitted in partial fulfillment of the requirements for the degree of MASTER OF SCIENCE
in MICROBIOLOGY

Montana State University

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Abstract:

BALB/c mice varied in their ability to make detectable antibody responses to cell surface determinants of *Candida albicans* depending upon the antigen preparation and the immunization schedule used. IgM was the major class of mouse antibody responsible for yeast cell agglutination. Various inbred strains of mice injected with a ribosomal fraction from *C. albicans* yeast cells produced low agglutinin (16) and high passive cutaneous anaphylaxis (PCA, 512) titers. Agglutinin activity of the serum may be due to an antibody class other than IgM because absorption of the serum with anti-mouse IgM did not affect the agglutinin activity. Also, the agglutinins appeared to be heat labile. Evidence which indicates IgE is responsible for the PCA reactions includes: (1) immune serum heat-treated at 56°C for 4 h lost its ability to elicit PCA reactions; and (2) loss of PCA activity was achieved by treating the immune serum with anti-mouse IgE. Although absorption of immune serum with *C. albicans* cells resulted in loss of agglutinin activity and reduction of PCA activity, it appears the IgE and agglutinin responses are specific for different antigens because: (1) agglutinins were removed from the serum by absorption with sheep erythrocytes coated with a soluble extract (SE) containing cell surface determinants of yeast cells but the PCA titer was not reduced; (2) agglutinins, but not IgE antibodies, were removed from the serum by affinity chromatography using SE-bound Sepharose beads; and (3) PCA reactions could not be provoked by the SE preparation.

IgE responses specific for *Candida* ribosomal antigens also occurred in mice injected intraperitoneally or subcutaneously with yeast cells. Although IgE against *C. albicans* strain 9938 was specific for ribosomal antigens from *C. albicans* strain 394 and a strain of *C. tropicalis*, no cross reactivity was detected against similar antigen preparations from *C. krusei* and *Saccharomyces cerevisiae*. IgE responses were also detected in mice with experimental candidiasis.

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Approved:

Jim E. Cutler
Chairperson, Graduate committee

Norman D. Reed
Head, Major Department

Michael Malone
Graduate Dean

MONTANA STATE UNIVERSITY
Bozeman, Montana

September, 1982

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Date

September 14, 1982

ACKNOWLEDGEMENTS

I am deeply grateful to all the members of the Microbiology Department for their support during my graduate studies. I am particularly thankful to Dr. Jim E. Cutler, my major advisor, for his patience and guidance.

I would also like to thank Drs. Sandra Ewald, Cliff Bond, and Norman Reed for their useful suggestions concerning my research work.

I must not overlook the helpful activities of Ruth Lloyd, a fine technician and friend.

Lastly, I thank my wife, Chris, for tolerating my short temper and stubbornness during the preparation and completion of this thesis.

This work was supported by grants from the National Institutes of Health.

TABLE OF CONTENTS

	Page
VITA.....	ii
ACKNOWLEDGEMENTS.....	iii
TABLE OF CONTENTS.....	iv
LIST OF TABLES.....	vii
LIST OF FIGURES.....	viii
ABSTRACT.....	ix
INTRODUCTION.....	1
MATERIALS AND METHODS.....	9
Organisms and Culture Conditions.....	9
Experimental Animals.....	9
Heat Killing of Yeasts.....	10
Serum Collection.....	10
Slide Agglutination Titer.....	10
Indirect Fluorescent Antibody Assay.....	11
Sonicated Yeast Cell Preparation.....	13
Cell Wall Fraction Preparation.....	13
<u>C. albicans</u> Soluble Extract Preparation.....	14
Ribosomal Fraction Preparation and Animal Injection.....	15
Treatment of Mouse Immune Sera with Anti-Mouse Isotypes.....	16
Absorption of Mouse Immune Sera with <u>C. albicans</u>	16

	Page
Coating of Sheep Erythrocytes with SE and Serum Absorption.....	17
Affinity Chromatography.....	18
Passive Cutaneous Anaphylaxis (PCA).....	19
RESULTS.....	21
Induction of Agglutinin Responses in Mice.....	21
Agglutinin Responses of Young and Older Female and Male Balb/c Mice.....	21
Heavy Chain Isotype Determination of <u>Candida</u> Agglutinins.....	24
<u>C. albicans</u> Ribosomal Fraction-Induced Agglutinin Responses in Mice.....	24
Passive Cutaneous Anaphylaxis Reactions in Rats.....	27
Identification of the Antibody Class Responsible for the PCA Reactions.....	31
Appearance of Agglutinins and IgE Responses During Immunization of Mice with <u>Candida</u> Ribosomal Fractions.....	31
Absorption of Immune Serum with <u>Candida</u> Cells: Effects on the PCA and Agglutinin Activity.....	32
Evidence That Agglutinin and IgE Responses Are Specific for Different <u>Candida</u> Antigens.....	32
IgE Responses in Mice Elicited by Whole Cells of <u>C. albicans</u>	36
Inability of Other Yeasts to Induce Anti- <u>C. albicans</u> IgE Responses.....	36

	Page
Specificity of Anti- <u>C. albicans</u> IgE Antibodies for Ribosomal Fractions From Other Yeasts.....	41
IgE Production in Mice with Experimental Candidiasis.....	41
DISCUSSION.....	45
LITERATURE CITED.....	56

LIST OF TABLES

Table	Page
1. Attempts to Induce Production of Anti- <u>C. albicans</u> Cell Surface Antibodies in Balb/c Mice.....	22
2. Whole Yeast Cell Immunizations for Production of <u>C. albicans</u> Agglutinin Responses in Balb/c Mice.....	23
3. Agglutinin Responses of Young and Older Female and Male Balb/c Mice Injected I.P. with HK <u>C. albicans</u>	25
4. Agglutinin Responses of Different Strains of Mice Injected with Ribosomal Fractions from <u>C. albicans</u> 9938.....	28
5. Passive Cutaneous Anaphylaxis (PCA) Reactions in Rats Elicited by Immune Sera From Mice Sensitized with <u>C. albicans</u> Ribosomal Fractions.....	29
6. Effect on PCA and Agglutinin Activity of Immune Serum Following Absorption with <u>Candida</u> Cells.....	34
7. Effect on PCA and Agglutinin Activity of Immune Serum Following Absorption with <u>Candida</u> Soluble Extract (SE)-Coated Sheep Erythrocytes and SE-Bound Sepharose Beads.....	35
8. IgE Responses Elicited in Balb/c Mice by Immunization with Whole Cells of <u>C. albicans</u>	37
9. Inability of Other Yeasts to Induce Anti- <u>C. albicans</u> IgE Responses in Balb/c Mice....	39
10. Ability of Other <u>Candida</u> Species to Induce IgE Responses in Balb/c Mice.....	40

LIST OF FIGURES

Figure	Page
1. Effect of Goat Anti-Mouse Heavy Chain Isotype Treatment on the Agglutination Titer of Anti- <u>C. albicans</u> Serum.....	26
2. Photograph of PCA Reactions on the Reverse Side of a Rat Skin.....	30
3. Agglutinin and IgE Responses of Balb/c Mice Immunized with <u>C. albicans</u> Ribosomal Fractions.....	33
4. Cross Reactivity of Anti- <u>C. albicans</u> IgE Antibodies for Ribosomal Fractions Prepared from Other Yeasts.....	42
5. Production of IgE Specific for <u>C. albicans</u> in Mice with Experimental Candidiasis.....	43

ABSTRACT

BALB/c mice varied in their ability to make detectable antibody responses to cell surface determinants of Candida albicans depending upon the antigen preparation and the immunization schedule used. IgM was the major class of mouse antibody responsible for yeast cell agglutination. Various inbred strains of mice injected with a ribosomal fraction from C. albicans yeast cells produced low agglutinin (16) and high passive cutaneous anaphylaxis (PCA, 512) titers. Agglutinin activity of the serum may be due to an antibody class other than IgM because absorption of the serum with anti-mouse IgM did not affect the agglutinin activity. Also, the agglutinins appeared to be heat labile. Evidence which indicates IgE is responsible for the PCA reactions includes: (1) immune serum heat-treated at 56°C for 4 h lost its ability to elicit PCA reactions; and (2) loss of PCA activity was achieved by treating the immune serum with anti-mouse IgE. Although absorption of immune serum with C. albicans cells resulted in loss of agglutinin activity and reduction of PCA activity, it appears the IgE and agglutinin responses are specific for different antigens because: (1) agglutinins were removed from the serum by absorption with sheep erythrocytes coated with a soluble extract (SE) containing cell surface determinants of yeast cells but the PCA titer was not reduced; (2) agglutinins, but not IgE antibodies, were removed from the serum by affinity chromatography using SE-bound Sepharose beads; and (3) PCA reactions could not be provoked by the SE preparation. IgE responses specific for Candida ribosomal antigens also occurred in mice injected intraperitoneally or subcutaneously with yeast cells. Although IgE against C. albicans strain 9938 was specific for ribosomal antigens from C. albicans strain 394 and a strain of C. tropicalis, no cross reactivity was detected against similar antigen preparations from C. krusei and Saccharomyces cerevisiae. IgE responses were also detected in mice with experimental candidiasis.

INTRODUCTION

Candida albicans is a yeast which commonly co-exists with other normal flora organisms on mucous membranes of humans (10,12). C. albicans usually does not cause disease in normal individuals, but under certain conditions of lowered host resistance, the fungus causes disease. These conditions include: (1) extreme youth, especially before establishment of normal flora; (2) physiological changes such as pregnancy, steroid treatment, or endocrine dysfunctions; (3) prolonged administration of antibiotics; (4) general debility of the host due to immunosuppressive treatment, neoplasias, or chronic disease; and (5) iatrogenic factors such as indwelling catheters, peritoneal dialysis, and surgical procedures.

Candida albicans can cause a variety of primary or secondary diseases all of which are termed candidiasis and include dermatomycosis, oral thrush, vaginitis, broncho-pneumonia, disease of the alimentary canal, and chronic mucocutaneous candidiasis (CMC). The organism can also invade deep tissues and cause endocarditis, meningitis, or septicemia (51,70).

As a consequence of infection of mucous membranes by C. albicans and of subclinical forms of candidiasis, the majority of individuals are hypersensitive to C. albicans

antigens, and antibodies specific for the fungus are commonly detectable in their sera (55). In fact, delayed-type hypersensitivity (DTH) reactions to this yeast are commonly used by clinicians to evaluate cell-mediated immune function.

Despite intense research, host resistance mechanisms against C. albicans have not been well defined. Innate barriers, including the skin and normal bacterial flora, are known to impede tissue invasion by the fungus and phagocytic cells, such as neutrophils and macrophages, have been implicated as being important for defense. Congenitally-thymic deficient (nude) mice are more resistant than their normal littermates (8) to an acute infection with C. albicans. This increased resistance may be due to enhanced phagocytic activity (8,54, our unpublished data). Lehrer and Cline (30) have shown that neutrophils have candidacidal activity and the myeloperoxidase system present in these cells has been shown to be lethal for Candida (29,32). Some patients with chronic granulomatous disease were found to be very susceptible to systemic candidiasis (31) and these patients also had myeloperoxidase system deficiencies.

Cell-mediated immunity (CMI) has been regarded as the

most important acquired specific defense against candidiasis. CMC patients usually show a depressed CMI response to C. albicans antigens while humoral immunity often appears intact (26,27). For example, Canales et al. (2) observed that an eleven year old patient with CMC was unreactive to mumps, trichophyton, streptokinase-streptodornase, PPD, coccidioidin, and histoplasmin skin test antigens. This patient also failed to respond to di-nitrochlorobenzene sensitization which confirmed the severity of defective CMI. Thymus-dependent immune responses were shown to be important for resistance against experimental cutaneous candidiasis in guinea pigs (62). Mice had increased resistance against an intravenous challenge of C. albicans after intracutaneous inoculations of viable yeast cells (17). This effect correlated with development of a positive DTH and presence of T-lymphocytes (41,22) and it did not occur in thymectomized, irradiated, and bone-marrow reconstituted animals which were vaccinated in the same manner.

Evidence for the importance of CMI in host resistance to candidiasis coincides also with observations made on the role of CMI in other fungal diseases such as coccidioidomycosis, histoplasmosis, cryptococcosis, and

dermatophytoses.

Many investigators have demonstrated that coccidioidomycosis is associated with CMI defects (3,44,4,50). During the course of this disease, patients may show depressed DTH reactions to dinitrochlorobenzene while complement-fixing antibody titers become greater (50). In experimental studies on histoplasmosis, protection can be transferred to normal mice via T-cells, but not serum, from mice immunized with ribosomes or live yeast cells of Histoplasma capsulatum (65,66). Diamond (9) showed that guinea pigs had increased resistance to cryptococcosis as compared to infected control animals when the animals were given immune cells from Cryptococcus neoformans-infected guinea pigs. Also, Lim and Murphy (35) reported that splenic-enriched T-cells, but not serum, from C. neoformans-infected mice could transfer immunity to normal mice as indicated by a reduction in number of yeast cells in tissues after viable yeast cell challenge. Other studies have also shown the importance of CMI in cryptococcosis (18,58). Cell-mediated immunity has also been implicated as important for local or partial immunity to dermatophyte infections (69,23,15,25).

In spite of the above indirect observations which

imply a correlation between CMI and resistance to candidiasis, other investigators have not found such a correlation (46,37,54,17). Pearsall et al. (46) were unable to show increased resistance in normal mice to C. albicans after adoptive transfer of lymphoid cells from yeast-vaccinated mice. This inability to transfer resistance occurred despite the fact that Candida-specific DTH developed in mice which received the Candida-sensitized lymphoid cells. Marra and Balish (37) showed that cutaneous hypersensitivity responses did not increase or correlate with the progression of disseminated candidiasis in mice. Congenitally thymic-deficient (nude) mice have been shown to be more resistant than normal littermates to acute disseminated candidiasis (8,54) whereas thymus reconstituted nude mice were as susceptible as normal mice (54). Thymectomized and irradiated mice have been shown not to be more susceptible to disseminated candidiasis than normal mice (17).

The role of antibody in resistance to fungal diseases is also a confusing issue. Many regard specific antibody as not important for resistance. The severity of disease in patients with coccidioidomycosis correlates well with a rise in complement-fixing antibody titers (50).

Patients with chronic mucocutaneous candidiasis usually show normal humoral immune responses despite anergy of cell-mediated immunity (26,27). Individuals with other forms of candidiasis also usually have normal antibody and defective CMI responses.

Although the above observations would militate against the importance of antibody in resistance to fungal infections, numerous investigators have demonstrated that antibody may play a protective role in experimental candidiasis (43,46,59,33,40,1). Moser and Dömer (40) showed that cyclophosphamide (CY) treatment of normal mice before cutaneous infection with C. albicans resulted in increased delayed-type hypersensitivity responses and decreased antibody responses to cytoplasmic antigens as compared to non-CY treated infected mice. The impaired ability of CY-treated mice to produce antibodies correlated with poor survival of these mice after intravenous challenge. Mice making agglutinin and precipitin responses after subcutaneous injection of ribosomal fractions from C. albicans were more resistant than control mice to chronic and acute infection (59,33). However, it is not possible to determine from these studies if humoral and/or cell-mediated immunity were

responsible for the increased resistance of vaccinated mice. Passive transfer to normal recipients of immune sera from mice vaccinated subcutaneously with sonicated C. albicans cells resulted in increased protection to an intravenous dose of viable cells (43). Intraperitoneal injection into normal animals of immune sera from mice vaccinated in the same manner resulted in increased resistance to intramuscular candidiasis (46).

Obviously, there is conflicting evidence concerning the importance of specific acquired immunity in host defense to candidiasis. Investigators have presented evidence which would argue for the importance of cell-mediated immunity in resistance to infection, but most of the evidence is indirect and not definitive. Other investigators have shown that antibodies may be important for protection. Evidence was also presented which suggests that neither humoral or cell-mediated immunity are important in host defense against candidiasis. Critical evaluation of the literature on the importance of humoral immunity in resistance to experimental candidiasis has led to some important questions. In the passive transfer experiments, did the immune sera actually contain anti-Candida antibodies? In most cases, investigators merely

collected sera from sensitized animals but did not specify antibody content. If antibodies were present, to which Candida antigens were they specific? Also, what antibody classes are produced in response to Candida antigens? Answers to these questions are essential if one is to correctly assess the role of humoral immunity in experimental candidiasis. It is the purpose of this study to answer some of these questions by characterizing the antibody responses of mice to C. albicans.

MATERIALS AND METHODS

Organisms and Culture Conditions

Candida albicans strain 9938 was from the Mycology Unit, Tulane Medical School, New Orleans, LA. C. albicans strain 394 was a gift from R. P. Morrison (University of Oklahoma, Norman, OK) and Candida tropicalis, Candida krusei, Cryptococcus neoformans, and Saccharomyces cerevisiae were from the Montana State University mycologic culture collection. Organisms were stored at 4° to 6°C on either potato flakes or corn meal agar slants.

All yeasts were grown in glucose (2%), yeast extract (0.3%), peptone (1.0%) broth (GYEP) at 37°C for 48 h under constant aeration by rotation of flasks at 160 rpm (Gyrotatory Incubator, New Brunswick Scientific, Edison, NJ).

Experimental Animals

BALB/c, BALB/cBYJ, DBA/1J, CBA/J, and B10.BR mice were obtained from Jackson Laboratories (Bar Harbor, ME). All mice were raised and maintained in our animal care facility in which bedding, cages, and water bottles were sterilized before use and the water was acidified (39). It had been previously determined in our laboratory that fecal specimens from mice were negative for Candida albicans and sera from normal mice do not contain de-

tectable C. albicans agglutinins or precipitins.

Heat Killing of Yeasts

Broth cultures were centrifuged and the yeast cells were washed three times with 0.15 N NaCl (saline). The washed cells were placed in a 70°C water bath for 20 min and killing was confirmed by streaking samples of the heated cells on nutrient agar plates and checking for growth after 24 h at 37°C.

Serum Collection

Mice were bled from either the tail artery or the retroorbital venous plexus and the blood was allowed to clot overnight at 4°C before the serum was removed. Sera were used immediately or stored at -20°C.

Slide Agglutination Titer

Sera from immunized animals were diluted 2-fold in saline and 25 ul of each diluted sample were mixed with an equal volume of a heat-killed yeast suspension in saline (1×10^8 cells/ml) on a glass slide. The cells and sera were mixed by rotating the slide by hand for 3 min. The presence or absence of agglutinated yeast cells was assessed by microscopic examination at 100x and the agglutination titer was expressed as the reciprocal of the

highest serum dilution showing positive agglutination. The slide method was chosen over the more conventional tube agglutination method because of the ease and speed of the method and the small amount of serum needed to perform the test. Comparison of the two methods showed that tube agglutination titers were always 4 times higher than slide agglutination titers.

Indirect Fluorescent Antibody Assay

Fluorescein isothiocyanate labeled rabbit anti-mouse gammaglobulin was prepared as described by Garvey et al. (16). BALB/c sera were collected by bleeding via the retroorbital venous plexus and allowing the pooled blood to clot overnight at 4°C. The gammaglobulin fraction of the serum was precipitated by the dropwise addition of one part saturated ammonium sulfate to two parts serum under constant stirring. The precipitate was dialyzed overnight against 0.01 M PBS, pH 7.2, and a 0.5 mg protein per ml stock solution of mouse gammaglobulin in 0.01 M PBS was prepared after determination of the protein concentration by 260-280 nm difference spectrometry. New Zealand white rabbits (Johnson's Bunny Farm, IN) were injected subcutaneously with 1 mg protein (2 ml) emul-

sified in 2 ml complete Freund adjuvant (CFA) on days 0 and 5. The same animals were injected on days 12 and 19 with 1 mg protein emulsified in 2 ml incomplete Freund adjuvant (IFA). Sera were collected from rabbits by cardiac puncture on day 26. The gammaglobulin fraction from the rabbit serum was obtained by ammonium sulfate fractionation and dialyzed overnight against 0.01 M PBS, pH 7.2. A 2.5 ml sample of a 2% rabbit gammaglobulin solution in 0.01 M PBS was added to 0.5 mg of fluorescein isothiocyanate (FITC, Sigma Chemical Co., St. Louis, MO) and the mixture was stirred overnight at 4°C. The FITC-gammaglobulin mixture was dialyzed against 0.0175 M phosphate buffer, pH 6.3, and uncoupled FITC was removed by anion exchange chromatography as previously described (16).

To perform the indirect immunofluorescent antibody assay (IFA), C. albicans yeast cells were bound to the bottom of Falcon 96 well MicroTest II tissue culture plates (Becton Dickinson Co., Oxnard, CA) by allowing the yeast cells to germinate in the wells for 1 h in the presence of tissue culture medium (TC 199, Microbiological Associates, Walkerville, MD) at 37°C. We previously determined that germinated cells avidly stick to plastic

whereas yeast cells do not. One-tenth milliliter samples of mouse immune sera to be tested for the presence of yeast cell surface specific antibodies were added to each well and the plate was incubated at room temperature for 30 min. After washing each well three times with saline, 25 ul of FITC labeled rabbit anti-mouse gammaglobulin were added to each well and the plate was incubated at room temperature for 20 min. The plate was washed three times with saline and observed under a light microscope equipped with illumination to detect FITC fluorescence.

Sonicated Yeast Cell Preparation

Sonicated yeast cells were prepared by the method of Mourad and Friedman (42). C. albicans strain 9938 was grown in GYEP for 48 h, washed three times, and suspended in saline to a concentration of 1×10^8 cells/ml. The cells were homogenized in a Braun homogenizer (Bronwell Scientific Co., Rochester, NY) with acid cleaned and sterilized glass beads 0.45 to 0.50 mm in diameter (Glasperlen, Bronwell Scientific Co.) for 3 to 5 min. The homogenate was defined as the sonicated cell suspension.

Cell Wall Fraction Preparation

C. albicans 9938 cell wall fractions were prepared

by the method described by Cutler et al. (7). Washed yeast cells were suspended in saline (20% aqueous suspension) and broken in a Sorval Ribi press at 35,000 psi. Ninety percent cell breakage was accomplished with this method. The broken cells were centrifuged at 800 x g for 30 min, the supernatant fluid was discarded, and the pellet washed three times with saline and lyophilized.

C. albicans Soluble Extract Preparation

A water soluble antigen was extracted from C. albicans by a hot phenol procedure previously described (68) and is referred to as soluble extract (SE). Yeast cells were suspended in distilled water to make a 20% (vol/vol) cell suspension and both the cells and a 90% phenol solution in water were brought to 65°C in a water bath. Equal volumes of the phenol and the suspension were mixed by swirling at 65°C for 30 min, cooled in an ice bath, and centrifuged at 180 x g for 45 min at 4°C. The aqueous phase was removed and replaced with an equal volume of heated (65°C) distilled water and the extraction procedure was repeated. The aqueous phase from both extractions were combined, dialyzed against distilled water for two days, and lyophilized.

Ribosomal Fraction Preparation and Animal Injection

Ribosomal fractions were prepared from yeasts by a modification of the procedures of Rubin (56) and Levy et al. (59). Yeast cells were grown in GYEP at 37°C for 48 h. The cells were harvested by centrifugation at 4°C (500 x g), washed three times with cold tris (hydroxymethyl) aminomethane-hydrochloride 0.01 M buffer containing 0.1 M NaCl and 0.03 M MgCl, pH 7.4 (TSMB), and resuspended in cold TSMB (75% aqueous suspension). The cell suspension was passed through a Sorval Ribi press two times at 35,000 psi. The broken cells were centrifuged at 26,000 x g for 20 min at 4°C and the supernatant fluid was removed and centrifuged through 4 ml of 5% ammonium sulfate and 15% sucrose in TSMB at 105,000 x g for 6 h at 4°C in a Beckman model L-2 Ultracentrifuge and SW 27 rotor (Beckman Instruments Inc., Irvine, CA). The pellet, defined as the crude ribosomal fraction, was resuspended in 0.01 M phosphate buffered saline (PBS), pH 7.4, and the protein concentration determined by 260-280 nm absorbancy in a spectrophotometer. The crude ribosomal fraction was diluted to a concentration of 1 mg protein/ml in 0.1 M PBS and stored at -70°C.

Mice were injected subcutaneously behind the head

with 100 ug ribosomal fraction protein emulsified in 1 ml complete Freund adjuvant (CFA) on day 0. The animals received booster injections on days 16 and 36 with 100 ug protein emulsified in incomplete Freund adjuvant (IFA) and sera were collected on day 57.

Treatment of Mouse Immune Sera with Anti-Mouse Isotypes

Attempts were made to remove mouse antibodies specific for C. albicans 9938 antigens by treating the immune sera with various anti-mouse heavy chain isotypes. One hundred microliters of immune serum were added to 100 ul of either goat anti-mouse IgG₁, IgG₂, IgM, IgA (Meloy, Springfield, VA) or IgE (Miles Laboratories, Elkhart, IN) in a 1.5 ml Eppendorf centrifuge tube (VWR, Seattle, WA). The mixture was incubated at 4°C overnight, centrifuged at high speed (Eppendorf centrifuge, Brinkman Instruments, Westbury, NY) for 5 min to pellet the precipitate, and the absorbed supernatant serum was removed.

Absorption of Mouse Immune Sera with C. albicans

Heat killed C. albicans cells were centrifuged to obtain a 0.1 ml wet pellet volume. The cells were suspended in 0.3 ml of mouse immune serum, incubated at

room temperature for 10 min, and centrifuged at 500 x g for 5 min. The absorbed serum was removed (0.3 ml) and re-absorbed with another 0.1 ml pellet of heat killed cells. The suspension was centrifuged at 500 x g for 5 min and the twice absorbed serum was collected.

Coating of Sheep Erythrocytes with SE and Serum Absorption

Six milliliters of a stock sheep erythrocyte suspension (Colorado Serum Co., Denver, CO) were pelleted by centrifugation at 250 x g for 5 min. The cells were washed three times with saline and centrifuged at 250 x g for 5 min to obtain a 0.5 ml packed volume of cells. The pellet was suspended in 1.0 ml of SE (1 mg/ml) and 1.0 ml of a 0.1% chromium chloride solution. The mixture was incubated at room temperature for 15 min, the cells were washed three times, and a final 10% (vol/vol) SRBC suspension was made in saline. To absorb immune sera, 0.5 ml of the 10% SRBC-SE suspension was added to a 1.5 ml plastic Eppendorf centrifuge tube, centrifuged in an Eppendorf centrifuge for 2 min and the supernatant fluid removed. Two hundred fifty microliters of immune serum were added to the tube, the cells suspended, and the mixture incubated for 10 min at 4°C. The cells were

pelleted by centrifugation and the supernatant serum was removed and reabsorbed with a fresh pellet of SRBC-SE. The twice absorbed serum was tested for yeast cell agglutination and PCA activity. As a control, immune serum was absorbed with sheep erythrocytes treated as above but without SE.

Affinity Chromatography

An affinity chromatography column was prepared by coupling C. albicans soluble extract antigens (SE) to Sepharose beads as described by Poor and Cutler (48). Sepharose 6B (Pharmacia Fine Chemicals, Piscataway, NJ) was washed five times with distilled water and centrifuged at 50 x g for 5 min. A 5.0 ml packed volume of beads was suspended with 5.0 ml of 0.5 M bicarbonate buffer (pH 11) plus 0.5 ml divinyl sulfone (Aldrich Chemical Co., Milwaukee, WI). The suspension was incubated for 70 min at 37°C under constant mixing by rotating at 150 rpm (incubator shaker), followed by washing in distilled water. A 4 ml packed volume of activated beads was added to 2 ml of a SE solution (15 mg SE per ml in 0.5 M bicarbonate buffer, pH 10) and incubated overnight at 22-24°C. The SE-coupled beads were collected by centrifugation, washed in distilled water,

suspended in 5 ml of 0.5 M bicarbonate buffer (pH 8.5) plus 0.1 ml beta-mercaptoethanol (J.T. Baker Chem. Co., Phillipsburg, NJ) and incubated at 22-24°C for 2 h. The beads were washed in distilled water and packed into a 1.0 ml plastic syringe (Becton, Dickonson and Co., Rutherford, NJ). After the column was equilibrated with 0.01 M PBS (pH 7.2) at 22-24°C, 0.5 ml of mouse immune serum was applied to the top of the column and eluted with 1.0 ml of 0.01 M PBS, pH 7.2. The collected eluate was washed through the same column again. The twice-eluted serum was concentrated to 0.5 ml by negative pressure dialysis at 4°C. As a control, immune serum was passed through a column of Sepharose 6B beads treated as above but without SE (negative control). Concentrated sera were tested for yeast cell agglutination and PCA activity.

Passive Cutaneous Anaphylaxis (PCA)

A modified method of Ovary (45) was used. Two hundred gram Sprague Dawley outbred albino female rats (Holtzman, Madison, WI and Taconic Farms, Germantown, NY) were anesthetized by an intraperitoneal injection of 3.6% chloral hydrate in saline (0.1 ml per 10 grams body weight) and hair removed from the back of the animals with

electric clippers. One-tenth milliliter of each two-fold serial dilution of serum was injected intradermally into the backs of the animals with a 27 gauge needle (Luer-Lok, Becton Dickonson, Rutherford, NJ). The rats were again anesthetized with chloral hydrate 48 h later and injected intravenously (tail vein) with 2 ml of an antigen preparation containing 1 mg protein of ribosomal fraction (1 ml) mixed with 1 ml of 2% Evans blue (Allied Chemical, Morristown, NJ). The PCA reactions were allowed to develop for 30 min, the animals were sacrificed and the skin reflected for observation of results. The PCA titer was expressed as the reciprocal of the highest dilution of serum which induced a discrete blue area greater than or equal to a 4 mm diameter.

RESULTS

Induction of Agglutinin Responses in Mice

BALB/c mice were injected intravenously (i.v.) intraperitoneally (i.p.), subcutaneously (s.c.), intramuscularly (i.m.), or intradermally (i.d.) with a variety of C. albicans strain antigens, including viable yeast cells, heat-killed cells, sonicated cells, and yeast cell wall fractions (Table 1). Sera collected on the days indicated and tested for the presence of antibodies by slide agglutination and indirect immunofluorescence were negative for antibodies specific for the cell wall of C. albicans. Injection protocols were finally developed, however, which did lead to induction of mouse antibodies specific for Candida cell surface determinants (Table 2). By 28 days following the first injection, all routes of inoculation resulted in the production of Candida agglutinins by at least 25% of the mice in each group.

Agglutinin Responses of Young and Older Female and Male BALB/c Mice

Because different groups of mice appeared to vary in their ability to make agglutinin responses when injected i.p. with heat killed (HK) C. albicans (Table 2), it was thought that the age and/or sex of the mice may affect the immune response to the fungus. BALB/c mice of different

Table 1. Attempts to induce production of anti-C. albicans cell surface antibodies in BALB/c mice.

Antigen	Inoculum	Route of Inoculation ^a	Days of Inoculation	Days of Sera Collection
Viable cells	1x10 ⁶	i.v.	0	4
	1x10 ⁶	i.v.	0	16
	2x10 ⁶	i.v.	0,23	15
	4x10 ⁸	i.m.	0	9,20,30
	5x10 ⁹	i.m.	0	4
Dead cells ^b	7x10 ⁷	i.v.	0,7,14,21	7,14,21,28
	2.8x10 ^{8c}	i.p.	0,15,36	64
Sonicated cells	2x10 ⁷	s.c.	0,5,12,20,30,40	14,21,28,35,47
Cell wall fraction	100ug	i.v.	0	4
	100ug	i.m.	0	4
	100ug	i.d.	0	4

^a i.v., intravenous; i.m., intramuscular; i.d., intradermal; i.p., intraperitoneal; s.c., subcutaneous.

^b C. albicans heat killed at 70°C for 20 min.

^c 0.1 ml volume of cells mixed with an equal volume of CFA.

Table 2. Whole yeast cell immunizations for production of C. albicans agglutinin responses in BALB/c mice.

HK ^a <u>C. albicans</u> inoculum	Route of Inoculation ^c	Days of Inoculation	# Positive ^d /total (%)
2x10 ⁷	i.v.	0,7,14,21	2/5(40)
4x10 ⁷	i.p.	0,7,14,21	3/3(100)
4x10 ⁷	i.p.	0,7,14,21	6/10(60)
4x10 ⁷	i.p.	0,7,14,21	17/64(27)
1x10 ⁷	s.c. ^b	0,7,14,21	2/5(40)

^a Heat-Killed.

^b 0.1 ml volume of cells mixed with an equal volume of CFA on days 0 and 7 and IFA on days 14 and 21.

^c i.v., intravenous; i.p., intraperitoneal; s.c., subcutaneous.

^d Number of mice producing agglutinins.

ages and sexes were injected i.p. with 4×10^7 HK C. albicans weekly for 4 weeks. Sera collected on the fifth week and titered for agglutinins revealed that all mice made agglutinin responses (Table 3). However, the older female mice tended to make greater responses than the other groups. These results did not explain the variability of the agglutinin responses shown in Table 2.

Heavy Chain Isotype Determination of Candida Agglutinins.

Immune sera from mice immunized i.p. with C. albicans were pooled and absorbed with goat anti-mouse heavy chain isotypes. Anti-mouse IgM absorption of the serum resulted in almost a 94% reduction in agglutination titer (Fig. 1). Anti-mouse IgG₁, IgG₂, and IgA treatment showed the same effect as the normal mouse serum control treatment. These data indicate that IgM is the major class of mouse antibody present in the immune serum which causes agglutination of C. albicans.

C. albicans Ribosomal Fraction-Induced Agglutinin Responses in Mice

Recently, Segal et al. (59) reported the induction of agglutinin responses in mice by immunizing with ribosomal fractions from C. albicans. Because of the difficulty

Table 3. Agglutinin responses of young and older female and male BALB/c mice injected i.p. with HK C. albicans.

BALB/c group ^a	# Positive ^b /total	Agglutinin Titer ^c	
		Range	Average
1	5/5	2-64	18
2	5/5	4-64	22
3	5/5	8-64	26
4	5/5	32-128	83

^a 1, males 21 to 28 days old; 2, females, 21 to 28 days old; 3, males, 56 to 63 days old; 4, females, 56 to 63 days old.

^b Number of mice producing agglutinins.

^c Expressed as the reciprocal of the highest serum dilution showing yeast cell agglutination.

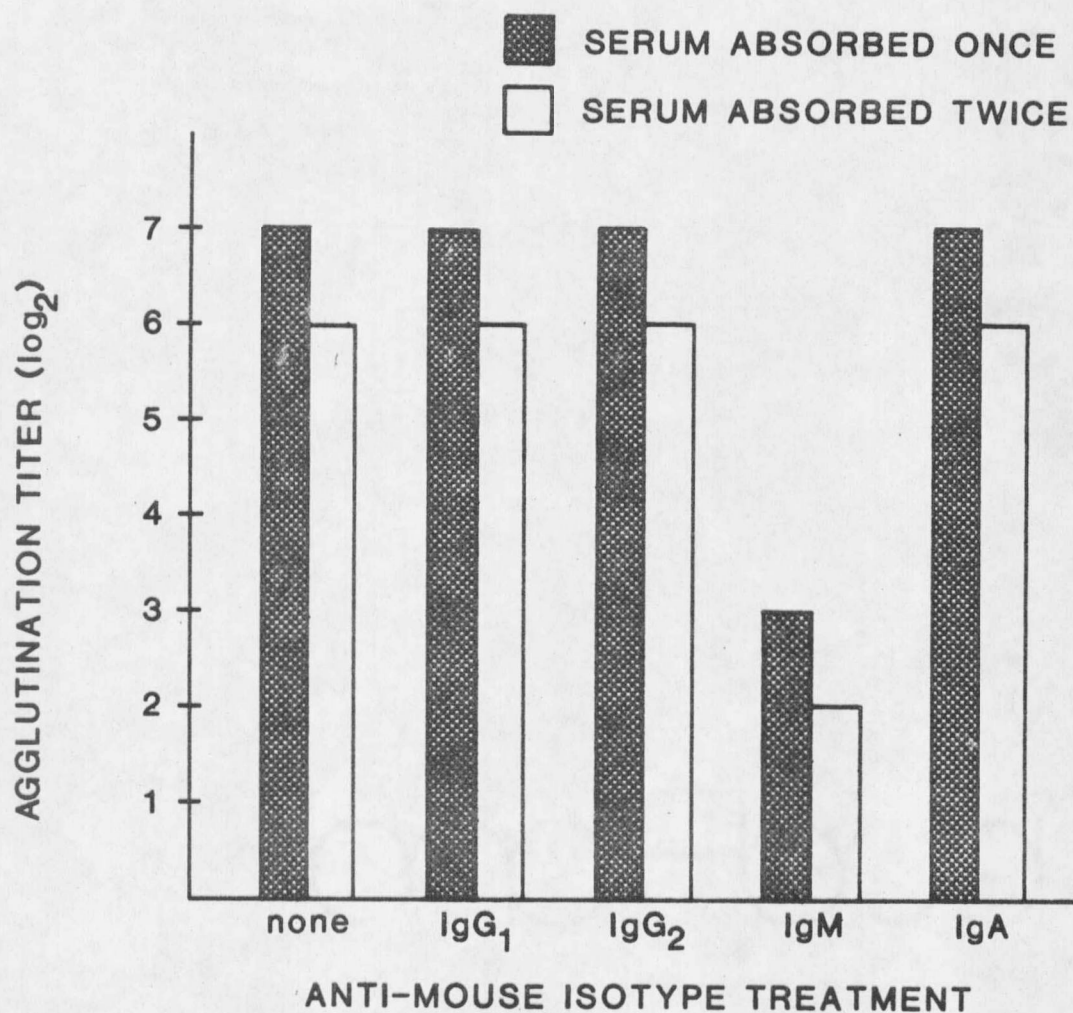


Figure 1. Effect of goat anti-mouse heavy chain isotype treatment on the agglutination titer of anti-*C. albicans* serum. Balb/c mouse immune serum was absorbed with an equal volume of either anti-mouse IgG₁, IgG₂, IgM or IgA at 4°C overnight and titered for *C. albicans* agglutinins. Normal mouse serum (NMS) was used as a control treatment.

encountered in inducing such agglutinin responses by more traditional sensitization protocols (Table 1), the ribosomal procedure was tested in different strains of mice. The animals were injected subcutaneously on day 0 with the C. albicans 9938 ribosomal fraction-adjuvant emulsion. Booster injections of ribosomal-adjuvant preparations were given on days 16 and 36. Sera collected on day 57 showed the presence of Candida agglutinins in some of the animals (Table 4). All strains made similar responses, but the titers were lower than sera from mice injected i.p. with whole cells (Table 3).

Passive Cutaneous Anaphylaxis Reactions in Rats

Immune sera from ribosomal fraction-immunized mice sometimes were found to lose yeast cell agglutinating activity after heating at 56°C for 30 to 120 min. To determine if the sera contained IgE antibodies, which are heat labile (19), passive cutaneous anaphylaxis (PCA) experiments were performed in rats. Whereas less than 50% of the mice showed evidence of agglutinins in their sera following ribosomal immunizations (Table 4), all but one of these sera gave positive PCA reactions in rats (Table 5). An example of a PCA reaction is shown in Fig. 2.

Table 4. Agglutinin responses of different strains of mice injected with ribosomal fractions from C. albicans 9938.

Mouse strain	Haplotype	# Positive ^a /total	Agglutination Titer ^b	
			Range	Average
BALB/c	d	5/10	2-8	5
DBA/1J	q	5/9	2-8	4
CBA/J	k	3/10	2	2
BALB/cBYJ	d	5/10	4-16	8
B10.BR	k	3/10	2-4	3

^a Number of mice producing agglutinins.

^b Expressed as the reciprocal of the highest serum dilution showing yeast cell agglutination.

Table 5. Passive cutaneous anaphylaxis (PCA) reactions in rats elicited by immune sera from mice sensitized with C. albicans ribosomal fractions.

Immune serum source ^a	Haplotype	# Positive ^b /total	PCA Titer ^c	
			Range	Average
BALB/c	d	10/10	4-512	111
DBA/1J	q	9/9	8-128	56
CBA/J	k	10/10	32-256	104
BALB/cBYJ	d	5/5	2-128	45
B10.BR	k	9/10	16-256	74

^a C. albicans agglutinating and nonagglutinating sera from various mouse strains sensitized with C. albicans ribosomal fractions.

^b Number of sera positive for PCA reactions.

^c Expressed as the reciprocal of the highest serum dilution showing a positive reaction.

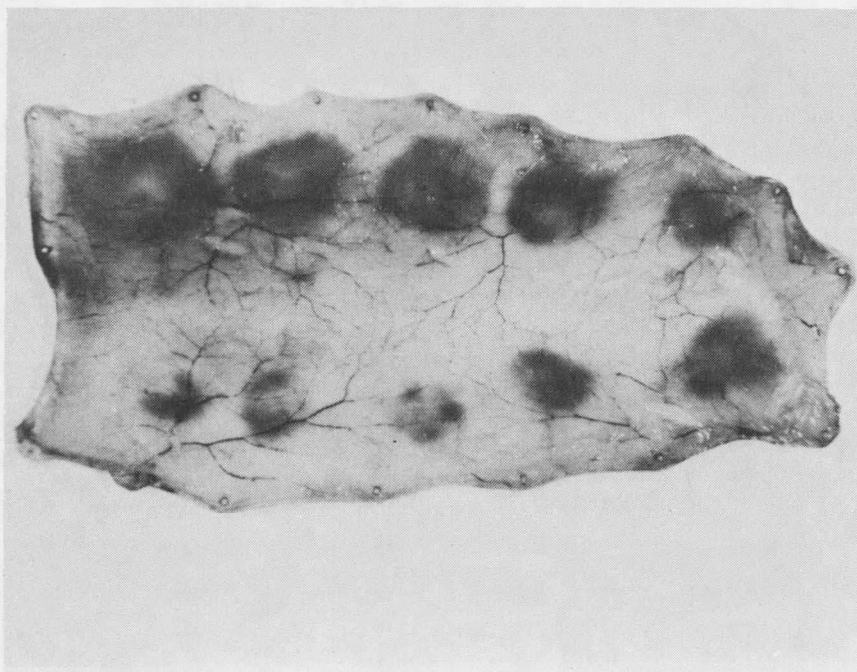


Figure 2. PCA reactions on the reverse side of a rat skin. A serum sample from a Balb/c mouse sensitized to C. albicans ribosomal fractions was serially diluted 2-fold in saline and 0.1 ml volumes were injected intradermally at different sites along the back of the rat. Forty eight hours later, 1 mg of C. albicans ribosomal fraction protein in 1% Evans blue (2 ml) was injected into a tail vein and the reactions were allowed to develop for 30 min. The above skin shows a PCA titer of 512 (largest reaction was due to undiluted serum).

Identification of the Antibody Class
Responsible for the PCA Reactions

Ishizaka and Ishizaka (19) reported that the mast cell sensitizing activity of mouse IgE, but not IgG, is lost when immune sera are heat treated at 56°C for 2 to 4 h. Serum from mice immunized with C. albicans ribosomal fractions and containing a PCA titer of 512 showed complete loss of PCA activity upon heating at 56°C for 4 h.

Immune serum was also treated with goat anti-mouse heavy chain isotypes in an attempt to remove the PCA activity. The PCA titer of the serum was not affected following absorption with an equal volume of either anti-mouse IgA, IgM, IgG₁, IgG₂, or normal mouse serum at 4°C overnight. A single absorption with anti-mouse IgE (anti-epsilon), however, eliminated the PCA activity.

These data indicate that the rat mast cell sensitizing antibody is IgE.

Appearance of Agglutinins and IgE Responses
During Immunization of Mice with Candida
Ribosomal Fractions

Five BALB/c female mice were immunized subcutaneously with the C. albicans 9938 ribosomal fraction-adjutant emulsion on days 0, 16, and 36. Sera were individually collected each week and titered for yeast cell

agglutinins and IgE responses (PCA reactions in rats). Whereas IgE antibodies were detected the second week of the immunization schedule, agglutinins were not apparent until the fourth week (Fig. 3). Also, IgE responses tended to increase during the immunization period while the agglutinin responses remained constant.

Absorption of Immune Serum with Candida
Cells: Effects on the PCA and Agglutinin Activity

Immune sera from BALB/c mice immunized with C. albicans ribosomal fractions were pooled, absorbed with heat killed Candida cells, and tested for agglutinin and PCA activity. Yeast cell absorption resulted in the abrogation of the agglutinin activity and significantly reduced the PCA activity of the immune serum (Table 6).

Evidence that Agglutinin and IgE Responses
are Specific for Different Candida Antigens

Sheep erythrocytes coated with the soluble extract (SE) antigen preparation from the cell wall of C. albicans were found to agglutinate when mixed with mouse serum which contained agglutinins to the fungus. Pooled BALB/c immune serum containing yeast cell agglutinins and IgE lost only the agglutinins when the serum was absorbed twice with SE-coated sheep erythrocytes (Table 7).

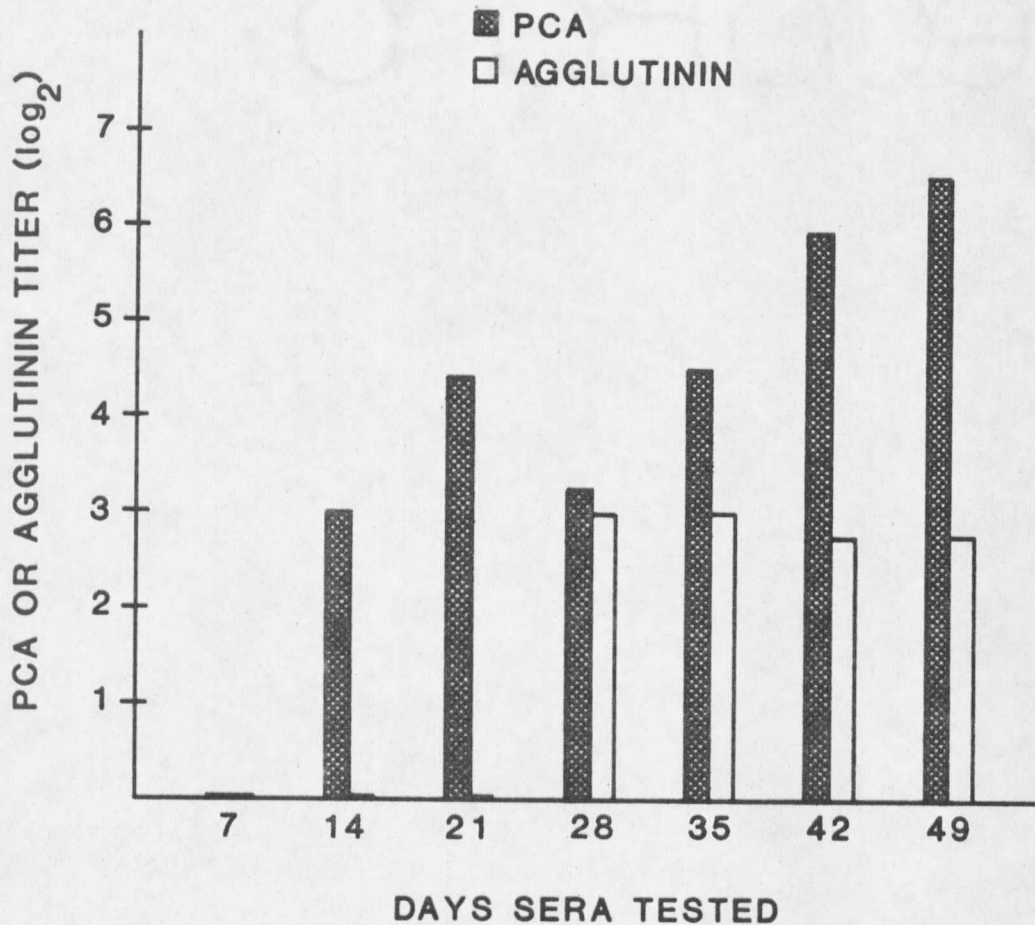


Figure 3. Agglutinin and IgE responses of Balb/c mice immunized with *C. albicans* ribosomal fractions. Five female mice were injected subcutaneously on days 0, 16, and 36 with 1.0 mg protein in CFA or IFA. Sera were collected and tested weekly for agglutinin and PCA activity. Each bar represents the average titer of those mice making agglutinin and/or IgE responses for the period tested.

Table 6. Effect on PCA and agglutinin activity of immune serum following absorption with Candida cells.

Serum Absorption Treatment	Agglutination Reaction ^a	PCA Titer
None	3+	128
Yeast cells ^b	0	16

^a Slide agglutination reaction scored on a scale from 0 to 4+.

^b C. albicans heat killed at 70°C for 20 min.

Table 7. Effect on PCA and agglutinin activity of immune serum following absorption with Candida soluble extract (SE) coated sheep erythrocytes and SE-bound Sepharose beads.

Serum Absorption Treatment	Agglutination Reaction ^a	PCA Titer
SRBC ^b (control)	3+	64
SE-coated SRBC	0	128
Sepharose beads (control)	2+	64
SE-bound Sepharose	0	32

^a Slide agglutination reaction scored on a scale from 0 to 4+.

^b Sheep red blood cells.

Likewise, after the immune serum was passed through an affinity chromatography column containing Candida SE-coated Sepharose beads, yeast cell agglutinins were not detected while PCA activity was only slightly diminished (Table 7).

Also, attempts to provoke PCA reactions in rats with the SE, rather than the ribosomal, antigen preparation were unsuccessful.

These data indicate that the agglutinin response elicited in mice by the injection of Candida ribosomal fractions is specific for the soluble extract antigen whereas the IgE response is specific for an antigen(s) in the C. albicans ribosomal preparation.

IgE Responses in Mice Elicited by Whole Cells of C. albicans

The majority of BALB/c mice which were immunized intraperitoneally or subcutaneously with heat killed C. albicans 9938 produced IgE responses (Table 8). However, no IgE responses were detectable in mice immunized by the intravenous route.

Inability of Other Yeasts to Induce Anti-C. albicans IgE Responses

To determine if other yeasts would induce IgE

Table 8. IgE responses elicited in BALB/c mice by immunization with whole cells of C. albicans.

Route of Inoculation	# of Yeast Cells ^a	# PCA Positive/total
i.v.	2×10^7	0/5
i.p.	4×10^7	4/5
s.c.	1×10^{7b}	5/5

^a C. albicans 9938 heat killed at 70°C for 20 min. Mice were immunized on days 0, 7, 14, 21, sera collected on day 28, and tested undiluted.

^b Cells were emulsified in CFA on day 0 and IFA on days 7, 14, and 21.

responses which cross react with C. albicans ribosomal antigens, mice were immunized with non-C. albicans yeasts, their sera collected, and PCA titers determined using the C. albicans ribosomal preparation to provoke the PCA reaction. Only sera from mice immunized with C. albicans were able to cause PCA reactions (Table 9). Sera from mice immunized with other Candida species or unrelated yeasts did not contain detectable IgE antibodies against C. albicans ribosomal antigens.

The absence of PCA reactions induced by sera from mice sensitized with yeasts other than C. albicans may be due to: (1) either the lack of an IgE response or a response below the level of detectability; or (2) production of IgE antibodies which do not cross react with C. albicans antigens. Sera obtained from mice immunized with C. tropicalis or C. krusei either produced a very low titer or an undetectable PCA reaction against their respective homologous ribosomal preparations (Table 10). These observations support the possibility that non-C. albicans yeasts induce only small or undetectable IgE responses.

Table 9. Inability of other yeasts to induce anti-C. albicans IgE responses in BALB/c mice.

Organism Injected ^a	# PCA Positive/total	PCA Titer ^c Range	Average
<u>C. albicans</u> 9938	3/5	1-16	11.0
<u>C. albicans</u> 9938 ^b	3/5	1-8	3.33
<u>C. albicans</u> 394	3/5	1-8	3.33
<u>C. tropicalis</u>	0/5	-	-
<u>C. krusei</u>	0/5	-	-
<u>Saccharomyces cerevisiae</u>	0/5	-	-
<u>Cryptococcus neoformans</u>	0/5	-	-

^a 1×10^7 organisms injected s.c. with CFA on day 0 and IFA on days 7, 14, and 21. Sera were collected on day 28.

^b Organism injected without adjuvants.

^c PCA reactions elicited by C. albicans ribosomal preparations.

Table 10. Ability of other Candida species to induce IgE responses in BALB/c mice.

<u>Candida</u> Species Injected ^a	PCA Eliciting Antigen	PCA Titer ^c
<u>C. krusei</u>	<u>C. krusei</u> RF ^b	0
<u>C. tropicalis</u>	<u>C. tropicalis</u> RF	2

^a 1×10^7 organisms injected s.c. with CFA on day 0 and IFA on days 7, 14, and 21. Sera were collected and pooled on day 28.

^b Ribosomal fraction.

^c Sera from five mice in each group were pooled and serially diluted.

Specificity of Anti-C. albicans IgE
Antibodies for Ribosomal Fractions from Other Yeasts

To determine the specificity of anti-C. albicans IgE antibodies, attempts were made to induce PCA reactions with ribosomal antigen preparations from other yeasts. Although IgE against C. albicans strain 9938 showed cross reactivity against ribosomal antigens from C. albicans strain 394 and from a strain of C. tropicalis, it showed better specificity for C. albicans strain 9938 ribosomal antigens (Fig. 4). No cross reactivity was detectable against similar antigenic preparations from C. krusei or Saccharomyces cerevisiae.

IgE Production in Mice with
Experimental Candidiasis

Sera from mice injected intravenously or intraperitoneally with various concentrations of viable C. albicans 9938 were able to induce positive PCA reactions (Fig. 5). Mice infected i.p. made an earlier IgE response than animals infected i.v. and the greatest and most prolonged response was seen in the group of mice injected i.p. with the largest number of yeast cells (2×10^7). Animals infected i.v. did not make detectable IgE responses until the fourth or fifth week. The PCA titers of positive sera

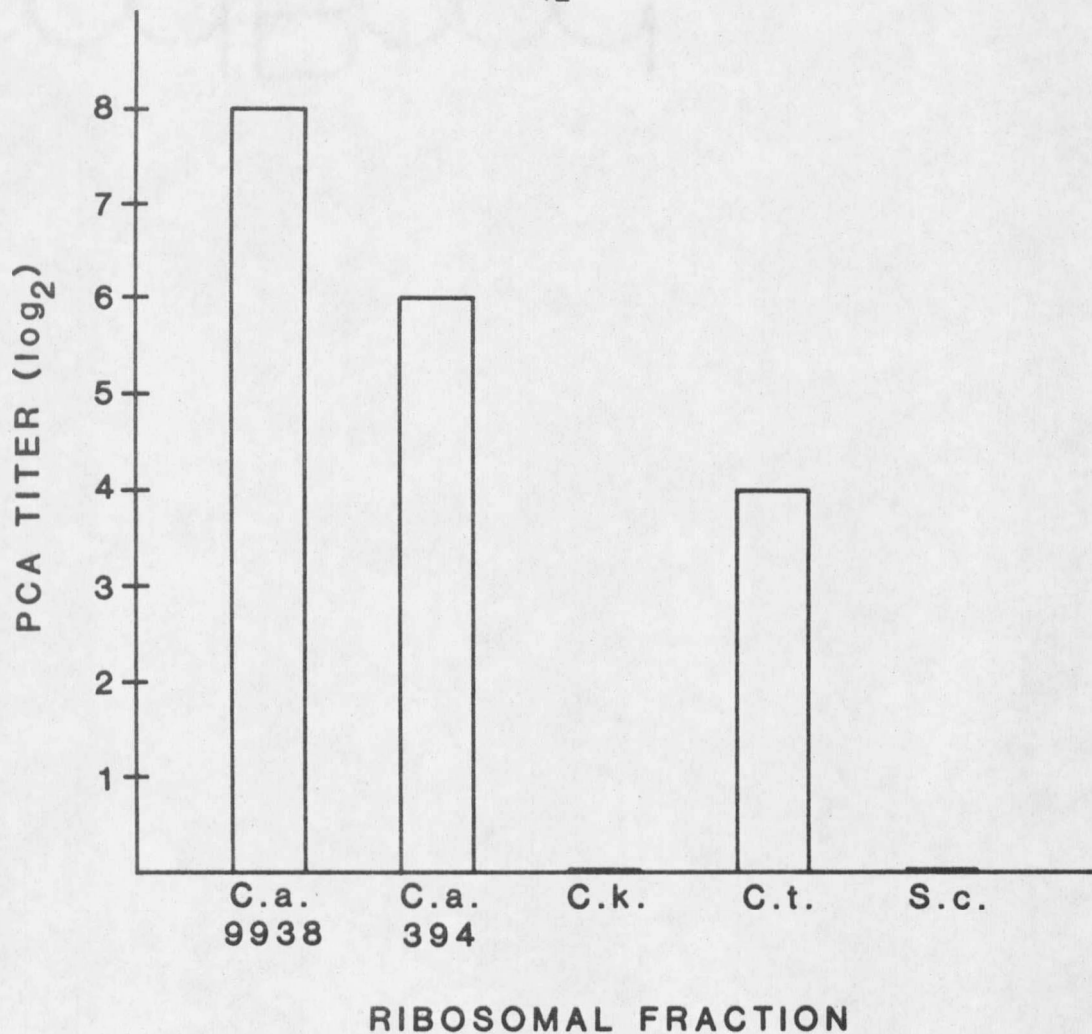


Figure 4. Cross reactivity of anti-C. albicans IgE antibodies for ribosomal fractions prepared from other yeasts. A PCA positive serum sample from Balb/c mice immunized with C. albicans 9938 ribosomal fractions was serially diluted 2-fold and injected intradermally into rats. Passive cutaneous anaphylaxis reactions were elicited with ribosomal fractions prepared from the different yeasts as indicated. C.a., Candida albicans; C.k., Candida krusei; C.t., Candida tropicalis; S.c., Saccharomyces cerevisiae.

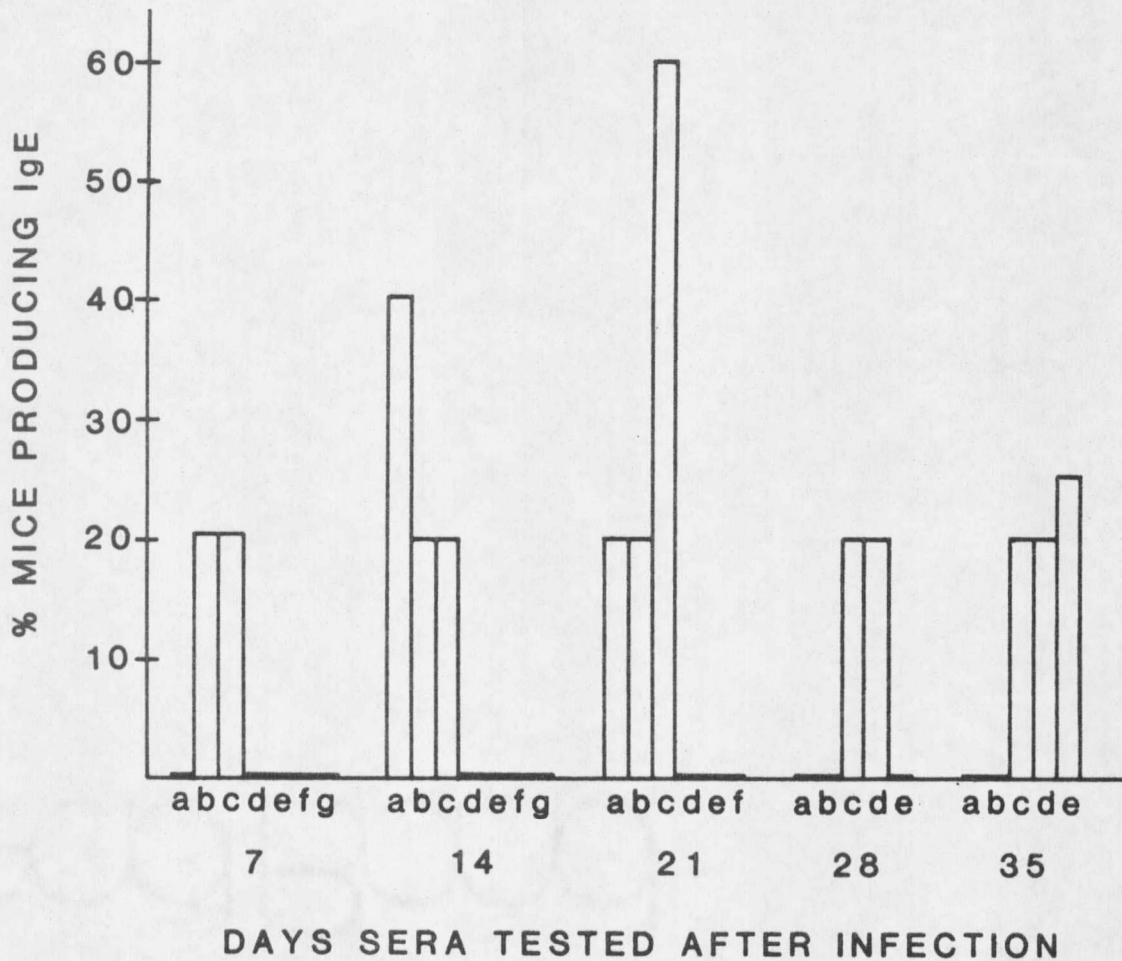


Figure 5. Production of IgE specific for *C. albicans* in mice with experimental candidiasis. Groups of Balb/c mice (5 per group) were infected by: (1) i.p. injection of 5×10^6 (a), 1×10^7 (b), or 2×10^7 (c) viable *C. albicans* 9938; or (2) i.v. injection of 2.5×10^5 (d), 5×10^5 (e), 1×10^6 (f), or 2×10^6 (g) viable yeast cells. Sera were collected weekly and tested for the presence of PCA eliciting antibodies specific for *C. albicans* ribosomal fractions. All mice in groups g and f died after the second and third weeks, respectively.

from all groups varied from 1 to 4 (\log_2) and two groups of mice (d and e) made the highest average PCA titer responses (\log_2 , 3.1 and 3.6, respectively, data not shown).

DISCUSSION

The importance of specific acquired immunity in host resistance to infection by Candida albicans is unclear. Cell-mediated immunity has been regarded as the most important specific acquired defense against candidiasis. However, investigations have shown that humoral immunity can play a protective role in host resistance. In order to correctly assess the role of antibodies in experimental candidiasis, more information is needed regarding the antibody responses of animals to Candida. Therefore, it was the purpose of this work to characterize the antibody responses of experimental mice to C. albicans.

Initial experiments were performed in an attempt to induce C. albicans cell surface specific antibody responses in mice. Despite using immunization protocols which result in agglutinin responses in our laboratory rabbits and guinea pigs, no agglutinin responses were detected in the mice. Also, sensitization methods of others (46,43,40) were unsuccessful in eliciting cell-surface specific antibody responses. Although Pearsall et al. (46) and Mourad and Friedman (43) could induce antibody responses in mice by immunizing the animals with sonicated Candida cells, it is not clear whether the responses were specific for cytoplasmic or cell surface

antigens. It is possible that other investigators in the past have only assumed that agglutinins were present in immune sera after C. albicans sensitization or have falsely reported Candida clumping factor (61) as yeast cell agglutinating antibody. We are also certain that our inability to induce detectable agglutinin responses in mice was not due to the use of a unique mouse strain (BALB/c) because other mouse strains and/or haplotypes responded in a similar manner (unpublished data).

Yeast cell injection protocols were finally developed, however, which did lead to induction of mouse antibody specific for Candida cell surface determinants (Table 2). Because the protocols specify weekly injection of yeast cells over a period of one month, it appears that prolonged immunization periods may be necessary for inducing C. albicans agglutinin responses in mice. It should also be pointed out that intravenous (i.v.) injection of yeast cells appeared to be a poor route of sensitization for production of agglutinins. This finding was interesting because i.v. injection of C. albicans is a good route of sensitization for inducing agglutinin responses in rabbits and guinea pigs. Also, different groups of BALB/c mice seemed to vary in their agglutinin

responses when immunized intraperitoneally (i.p.) with C. albicans cells and female mice eight to nine weeks old may make slightly greater responses than male mice of the same age or younger male and female mice, two to three weeks old.

Both IgG and IgM antibodies can cause agglutination of particulate antigens. We feel that IgM is the major class of antibody responsible for Candida agglutinating activity in immune sera from mice immunized with whole yeast cells because absorption of the serum with goat anti-mouse IgM removes the yeast cell agglutinins. Absorption with anti-IgG₁, IgG₂, and IgA does not affect the agglutinating activity of the serum.

Vaccination with crude ribosomal fractions has been shown to protect animals against experimental infections caused by bacteria (14,34,36,57), Histoplasma capsulatum (13) and Candida albicans (33). Because of the difficulty initially encountered in inducing candidal agglutinin responses in mice and because injections of C. albicans ribosomal fractions not only protect mice but also cause induction of agglutinins (59), ribosomal vaccines were made in an attempt to induce Candida agglutinins in our mice. Between 30 and 50% of the mice

made yeast cell surface antibody responses following immunization with the ribosomal preparation. The response was consistent in five different strains of mice and agglutinin titers were considerably lower than those produced in mice immunized with whole cells of C. albicans. Although it may seem unusual that the ribosomal preparation induces a yeast cell agglutinin response, investigators have shown that ribosomal preparations from bacteria will induce cell surface antibody responses in experimental animals (11,63). A possible explanation for this phenomenon is that the agglutinin response is elicited by nascent proteins in the ribosomal preparation which become constituents of the yeast cell surface. Also, because the ribosomal fraction is isolated by cell breakage and differential centrifugation, it is likely that cell wall polysaccharides and proteins are contaminants of the antigen preparation.

Agglutinins induced by ribosomal fractions appear to be different from those induced by whole cell immunization. Agglutinins against ribosomal antigens may be sensitive to heat at 56°C and agglutinin activity was unaffected by absorption of sera with anti-mouse IgM and other heavy chain specific antibodies. It would appear

that the agglutinating activity of the serum is not due to clumping factor because the clumping activity is induced by C. albicans sensitization and the activity is absent in normal mouse serum. Clumping factor has been shown to exist in normal rabbit serum and its activity subsides during active immunization with Candida antigens (61).

The possible heat instability of ribosomal antigen induced agglutinins led us to believe the yeast cell agglutination activity of immune serum was due to mouse IgE. IgG and IgM antibodies are heat stable whereas IgE antibodies have been shown to be heat labile (19). We were able to demonstrate that virtually all the sera from mice sensitized with C. albicans ribosomal antigens induced positive passive cutaneous anaphylaxis (PCA) reactions in rats when the ribosomal preparation was used as the PCA provoking antigen. The mast cell-sensitizing antibody responses were consistent in the five different strains of mice tested. Although guinea pig IgG antibodies against C. albicans have been shown to induce PCA reactions (67), we believe mouse IgE is responsible for the PCA reactions because: (1) serum loses its ability to provoke PCA reactions after heating to 56° for 4 h (a treatment which prevents IgE from sensitizing rat mast

cells); and (2) PCA activity is lost after serum absorption with goat anti-mouse IgE (anti-mouse IgG shows no effect). Immediate hypersensitivity and PCA reactions to C. albicans antigens have been reported in animal studies (24,21) and elevated serum anti-C. albicans IgE antibodies have been reported in patients with candidiasis (38). Also, immediate hypersensitivity reactions to Candida antigens have been reported in individuals with candidiasis and asthma (20,47,28,60).

Testing mouse sera weekly for the presence of agglutinins and PCA activity during the Candida ribosomal antigen immunization schedule demonstrated that IgE antibodies were detected in the sera during the second week of the immunization period, whereas agglutinins were not apparent until the fourth week. The earlier detection of IgE antibodies may be explained by the increased sensitivity of passive cutaneous anaphylaxis as compared to antibody detection by yeast cell agglutination. The IgE responses, however, tended to increase during the immunization schedule while the agglutinin responses remained constant.

Although absorption of immune serum containing yeast cell agglutinins and IgE antibodies with whole cells of

C. albicans completely removed the agglutinin activity, we were surprised that the PCA activity of the serum was significantly reduced by the absorption. It is possible that the IgE eliciting antigen in the ribosomal preparation is present on the yeast cell surface. Also, the IgE antibodies may cross react with a cell surface antigen or the antibodies may bind to the surface non-specifically. We have evidence which may indicate the IgE eliciting antigen is present on the yeast cell surface. BALB/c mice do make IgE responses specific for C. albicans ribosomal antigens (detected by PCA reactions) after intraperitoneal or subcutaneous injection of whole Candida cells. Our observations agree with those of Kabe et al. (21) who demonstrated that guinea pigs sensitized intraperitoneally with whole cells of C. albicans later develop immediate wheal and flare reactions after intradermal injection of Candida carbohydrate and protein antigens. It is interesting that no IgE responses were detected when the mice were immunized intravenously. It does not appear that the response is dose dependent because the number of cells injected i.v. (2×10^7) was between the number of cells injected i.p. (4×10^7) and s.c. (1×10^7). Because the yeast cells were not emulsified in Freund

adjuvant before intraperitoneal injection, it would also appear that the response is not dependent upon the use of adjuvants.

Although we are uncertain about the IgE-yeast cell surface interaction, we do believe the agglutinin and IgE responses are specific for different C. albicans antigens because: (1) yeast cell agglutination activity is removed from immune serum by absorption with sheep erythrocytes coated with a soluble extract (SE) antigen preparation from the cell wall of C. albicans. However, this treatment does not affect the PCA activity of the serum; (2) passage of the serum through a SE-bound Sepharose bead column abrogates the agglutinin activity whereas the PCA activity is only slightly diminished; and (3) the SE antigen preparation will not provoke PCA reactions in rats.

Mice injected subcutaneously with non-C. albicans yeasts (Table 10) did not make detectable IgE responses which cross react with C. albicans ribosomal antigens because sera from these mice were unable to provoke PCA reactions in rats. However, it is possible that C. albicans cells induce better IgE responses than other Candida species because sera from mice sensitized with C. tropicalis or C. krusei either produced a very low

titered or undetectable PCA reaction against their respective ribosomal preparation.

We are certain, however, that the IgE eliciting antigen in the ribosomal preparations is not unique to C. albicans. Although immune sera from mice immunized with C. albicans 9938 ribosomal antigens produce strong PCA reactions when the same ribosomal preparation is used as the PCA provoking antigen, weaker PCA reactions are elicited when ribosomal preparations from C. albicans strain 394 and C. tropicalis are used as the provoking antigen. However, PCA reactions could not be provoked by similar antigenic preparations from C. krusei or Saccharomyces cerevisiae.

Tang and Morelli (64) demonstrated immediate hypersensitivity reactions in mice against polysaccharide antigens from Cryptococcus neoformans after sensitization with viable yeast cells. Other studies have shown increased levels of serum IgE in patients with histoplasmosis (6), coccidioidomycosis (5), and systemic and vaginal candidiasis (38). Also, patients with chronic mucocutaneous candidiasis have demonstrated immediate hypersensitivity reactions to C. albicans antigens (28,60). Our studies indicate that mice with exper-

imental candidiasis do make IgE responses specific for C. albicans ribosomal antigens. Mice infected intraperitoneally made earlier, greater, and more prolonged responses than animals infected intravenously and the PCA titers of the immune sera were comparable to those from mice sensitized intraperitoneally with heat killed C. albicans (Table 9). Although we were unable to correlate IgE levels with disease severity, IgE antibodies could play a role in pathogenesis of candidiasis by: (1) coating the fungus with non-opsonizing antibody; and (2) inducing immediate hypersensitivity reactions in tissues of afflicted animals. Histamine release has been shown to suppress lymphocyte proliferation in vitro, delayed type hypersensitivity reactions in vitro (52), and migration inhibitory factor production (53). Release of other mast cell products at the site of infection could lead to diminished chemotactic responsiveness of neutrophils (49), cells which have been shown to be an important innate defense against C. albicans (8,29,32).

Our findings can be summarized as follows: 1. Although it is difficult to induce antibody responses specific for the cell surface of C. albicans by traditional sensitization procedures, injection of whole

cells by various routes over a prolonged period of time does lead to agglutinin responses (primarily IgM) in mice;

2. Mice immunized with crude ribosomal fractions from C. albicans make agglutinin and IgE responses. The two responses, however, are specific for different antigens;
3. The IgE antibodies do not cross react with ribosomal antigens from yeasts tested other than C. albicans and C. tropicalis;
4. Mice sensitized with heat killed C. albicans or infected with viable cells also make IgE responses specific for C. albicans ribosomal antigens.

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