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) agglu- tinins 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 Saccharomvces cerevisiae. IgE responses were also detected in mice with experimental candidiasis.

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Approved:

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Bozeman, Montana

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Date _September 14, 1982_

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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.

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. Congenically-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 susceptable 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 dinitrochlorobenzene 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 coccidioid-
omycosis 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 crypto-
coccosis (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 susceptable as normal mice (54). Thymectomized and irradiated mice have been shown not to be more susceptable 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 Domer (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^oC 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^oC for 48 h under

constant aeration by rotation of flasks at 160 rpm (Gyro-

tatory 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 <u>C. albicans</u> 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 $70^{\circ}C$ 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 $37^{\circ}C$.

Serum Collection

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

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 over-
night 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 concen-
tration 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 x 10^8 cells/ml. The
cells were homogenized in a Braun homogenizer (Bronwell
Scientific Co., Rochester, NY) with acid cleaned and ster-
ilized glass beads 0.45 to 0.50 mm in diameter (Glasper-
len, Bronwell Scientific Co.) for 3 to 5 min. The homo-
genate 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 sus-
pension) 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 supernatent 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. al-
bicans 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 $65^{\circ}C$ in a water bath.
Equal volumes of the phenol and the suspension were mixed by
swirling at $65^{\circ}C$ for 30 min, cooled in an ice bath, and
centrifuged at 180 x g for 45 min at $4^{\circ}C$. The aqueous phase
was removed and replaced with an equal volume of heated
($65^{\circ}C$) distilled water and the extraction procedure was
repeated. The aqueous phase from both extractions were com-
bined, 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 $37^{o}C$ for 48 h. The cells were harvested by centrifugation at $4^{o}C$ (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 $4^{o}C$ and the supernatent 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° 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 $-70^{o}C$.

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 re-
ceived 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 IgG_1, IgG_2, 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 $4^{\circ}C$ overnight, centrifuged at
high speed (Eppendorf centrifuge, Brinkman Instruments,
Westbury, NY) for 5 min to pellet the precipitate, and the
absorbed supernatent 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 sus-
pension (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 in-
cubated at room temperature for 15 min, the cells were
washed three times, and a final 10% (vol/vol) SRBC sus-
pension 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 supernatent 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 supernatent 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 bicarbonite 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 prep-
aration 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.), intra-
muscularly (i.m.), or intradermally (i.d.) with a vari-
ety 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 agglu-
tinins 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	1×10^6	i.v.	0	4
	1×10^6	i.v.	0	16
	2×10^6	i.v.	0,23	15
	4×10^8	i.m.	0	9,20,30
	5×10^9	i.m.	0	4
Dead cells[b]	7×10^7	i.v.	0,7,14,21	7,14,21,28
	2.8×10^{8c}	i.p.	0,15,36	64
Sonicated cells	2×10^7	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 <u>C. albicans</u> agglutinin responses in
BALB/c mice.

HK[a] C. albicans inoculum	Route of Inoculation[c]	Days of Inoculation	# Positive[d]/total (%)
2×10^7	i.v.	0,7,14,21	2/5(40)
4×10^7	i.p.	0,7,14,21	3/3(100)
4×10^7	i.p.	0,7,14,21	6/10(60)
4×10^7	i.p.	0,7,14,21	17/64(27)
1×10^7	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., sub-
cutaneous.

[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 vari-
ability 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 IgG_1, IgG_2, 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 anti-
body present in the immune serum which causes agglutin-
ation 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.