Partially Purified Antibodies Used in a Solid-Phase Radioimmunoassay for Detecting Candidal Antigenemia

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The development of a solid-phase radioimmunoassay procedure for the detection of *Candida albicans* antigens in serum of mice is described. Antibodies against *C. albicans* that were used in the radioimmunoassay procedure were partially purified from immune serum by a *C. albicans* antigen-coupled affinity column. Elution of anti-*C. albicans* antibodies from the column was by glucose and mannose; 4 mg of protein was recovered per ml, which contained 50% of the candidal agglutinin activity of immune serum. Also, 81% of the protein (partially purified antibody) recovered was adsorbed by whole *C. albicans* cells. Anti-*C. albicans* antibodies were either coupled to Sepharose 4B for use as the solid phase to bind candidal antigen in serum of infected animals, or radioiodinated (^{125}I) for use as a tracer molecule to bind to the candidal antigen solid-phase complex. Although control experiments indicated that at least 100 ng of candidal antigen should be present in a serum specimen for a positive radioimmunoassay test, candidal antigenemia was detected in 70.4% of infected mice even in cases where blood cultures for *C. albicans* were negative. With further refinement and adaptability to human serum, the radioimmunoassay test may become a helpful tool for use in the diagnosis of systemic candidiasis.

Recent evidence indicates that the incidence of disseminated candidiasis in hospitalized individuals is rising, yet the diagnosis of such cases continues to be a clinical challenge (10, 16). Because diagnosis by blood culture is unreliable (6, 9, 16, 19) and time-consuming (10, 18), attempts have been made to identify systemic candidiasis by the presence in the serum of antibodies to candidal antigens (17, 19, 20). Although some workers believe that there is diagnostic significance in detecting candidal agglutinins (14) and precipitins (5, 14) in patients' sera, others believe that these tests are of questionable value (7, 12). Major problems associated with interpreting results of these various serological tests include: individuals who have positive agglutinin and precipitin titers, yet are without evidence of disseminated candidiasis; individuals with disseminated disease but who are anergic; and the lack of standardized reagents used in the tests.

Another approach to diagnosing systemic candidiasis is to detect candidal antigens or other cell products in sera of infected patients. Advantages of this approach are that the tests would not be dependent upon the immune response of the host, and that presumably only patients with disseminated disease would shed antigen in the serum. Also, it may be possible that antigenemia could be detected in time for efficacious antifun-

gal therapy. A drawback may be that fungal antigens become cleared from the circulation too rapidly to allow detection. In 1974, Miller et al. (15) reported that by using gas-liquid chromatography candidal mannans could be detected in sera of patients with septicemia due to *Candida albicans*. More recently, by using a hemagglutination inhibition assay, Weiner and Yount (21) successfully detected mannan antigenemia in some patients with invasive forms of candidiasis.

The above workers have demonstrated that it may be possible to diagnose disseminated candidiasis through the use of sensitive assays for candidal antigens. In this paper we report the development of a radioimmunoassay (RIA) procedure using partially purified antibodies against *C. albicans* for detecting candidal antigen in sera of infected animals.

MATERIALS AND METHODS

Preparation of immune serum. *C. albicans* 9938 (obtained from the Mycology Unit, Tulane Medical School, New Orleans, La.) was grown in glucose (2.0%)-yeast extract (0.3%)-peptone (1%) broth at 37°C and aerated by rotating the culture vessel at 180 rpm (Incubator-shaker, New Brunswick Scientific, Edison, N.J.). After 48 to 72 h the organisms were harvested by centrifugation, washed three times with sterile 0.01 M phosphate buffer in 0.15 M NaCl, pH 7.2 (PBS), and resuspended to a concentration of about 4×10^8 yeast cells per ml. The fungal cells were

killed by heating at 62°C for 10 min. The heat-killed cells in suspension were emulsified in complete Freund adjuvant (Grand Island Biological Co., Grand Island, N.Y.) and injected subcutaneously into rabbits. Booster injections of heat-killed cells emulsified in incomplete Freund adjuvant were given on days 7 and 14. On day 24 the rabbits were bled, and the sera were titrated for *C. albicans* agglutinins.

Preparation of the soluble antigen. The soluble antigen (PE) was recovered from the aqueous phase of a hot-phenol extract of whole cells of *C. albicans* prepared as reported previously (4, 22).

Partial purification of anti-CA antibodies by affinity chromatography. An affinity column was prepared by coupling the antigen (PE) to Sepharose 6B (Pharmacia Fine Chemicals, Piscataway, N.J.) using divinyl sulfone as reported elsewhere (8), but with some modifications. Sepharose 6B was washed with distilled water, centrifuged at $51 \times g$ for 5 min, and washed again, and a 25-ml packed volume of the beads was suspended in 25 ml of 0.5 M carbonate buffer (pH 11) plus 2.5 ml of divinyl sulfone (Aldrich Chemical Co., Inc., Milwaukee, Wis.). The suspension was incubated for 70 min at 22 to 24°C under constant mixing by rotating at 120 rpm (Incubator-shaker), followed by washing in distilled water. A 20-ml packed volume of activated beads was added to 10 ml of PE solution (15 mg of PE per ml in 0.5 M carbonate buffer, pH 10) and incubated overnight at 22 to 24°C. After incubation the PE-coupled beads were collected by centrifugation, washed in distilled water, suspended in 25 ml of 0.5 M bicarbonate buffer (pH 8.5) plus 0.5 ml beta-mercaptoethanol (J. T. Baker Chemical Co., Phillipsburg, N.J.), and incubated at 22 to 24°C for 2 h. The beads were finally washed with distilled water and packed into a glass column (1.5 by 5.0 cm). After the column was equilibrated with PBS at 22 to 24°C, 5 ml of immune serum was applied to the top of the column and washed through with PBS. The eluted serum was washed through the same column again. The twice-eluted serum was collected and titrated for *C. albicans* agglutinins. As a control, immune serum was washed through a column of Sepharose 6B beads treated as above but without PE (negative control column), and the agglutinin titer of the eluate was determined. After the PE-coupled column was washed with 1 bed volume of PBS, anti-*C. albicans* (anti-CA) antibodies were eluted from the beads by first washing the column with 50 ml of 0.5 M glucose (Fisher Chemical, Fair Lawn, N.J.), then washing with 50 ml of 0.5 M mannose (J. T. Baker Chemical Co.). The eluates from both washes were concentrated individually by negative pressure dialysis at 4°C to a final volume of 2.5 ml, and the monosaccharides were removed by extensive dialysis against PBS at 4°C.

The concentrated and dialyzed eluates were titrated for anti-CA agglutinins, then pooled (partially purified antibody), concentrated to 2.5 ml, and retitrated. The degree of antibody purification was estimated by determining the amounts of protein (by the method of Lowry et al.; 13) in the immune serum and column-purified antibody. The percent of protein of the partially purified antibody that would bind to *C. albicans* was determined by absorbing the antibody with yeast cells and comparing the amount of free protein (13)

present before and after absorption. Agglutinin cross-reactivity in the immune serum was determined before and after partial purification of anti-CA antibodies against an additional strain of *C. albicans* (strain 2252, a gift from T. Mitchell, Duke University) and against *C. tropicalis*, *Saccharomyces cerevisiae*, *Cryptococcus neoformans*, and *C. laurentii* (all from Montana State University). The inhibitory activity of monosaccharides for partially purified antibody was tested by adding various amounts of glucose and mannose to the diluent (PBS) used in the agglutinin assay.

Iodination of antigen and purified antibodies. PE and partially purified anti-CA antibodies were iodinated using the chloramine T method of Greenwood et al. (11). Briefly, anti-CA antibodies were concentrated 10× by negative pressure dialysis (40 mg of protein per ml), and either 30 µl of this preparation or 10 µl of PE (10 mg/ml) was added to 100 µl of 0.5 M phosphate-buffered saline (pH 7.5) in a 1-ml glass vial; then 1 mCi of ^{125}I (Na^{125}I , New England Nuclear Corp., Boston, Mass.) was added to the mixture. Ten microliters of chloramine T (Eastman-Kodak, Rochester, N.Y.) (2 mg/ml in 0.05 M Na_3PO_4) was added to the reaction vessel, and after 2 min the reaction was stopped by the addition of 20 µl of sodium metabisulfite (2 mg/ml in 0.05 M Na_3PO_4). The labeled antigen or antibody was separated from unreacted ^{125}I by washing the contents of the reaction vessel through a Sephadex G-25 (Pharmacia) column (0.5 by 17.5 cm) with PBS at 2 ml/min. One-milliliter fractions were collected, and the amount of radioactivity in each fraction was determined (Beckman Biogamma, Beckman Instruments, Fullerton, Calif.). Under these conditions it was previously determined that both PE and anti-CA antibodies are excluded in the void volume of the G-25 column, while free iodine is retained. Fractions representing the labeled anti-CA antibodies or labeled PE were pooled, dialyzed against PBS for 48 h at 4°C, and stored at 4°C.

Preparation of solid-phase antibody. Partially purified anti-CA antibodies, prepared as described above, were coupled to Sepharose 4B (Pharmacia) with CNBr (Aldrich Chemical Co.) using a modification of a procedure published elsewhere (2, 3). Sepharose 4B was washed twice with distilled water, and 20 ml of packed beads was suspended in an equal volume of distilled water. Two grams of CNBr was added to the Sepharose beads; the reaction was maintained under constant stirring and at 20°C by the addition of ice. The pH was continuously monitored and kept at 11 by dropwise addition of 0.4 N NaOH. As previously reported (2), the reaction was complete when the pH remained constant (10 to 15 min); at that time the reaction was cooled to 4°C by the addition of ice. The activated Sepharose was washed with 7 to 10 volumes of cold 0.01 M borate buffer (pH 9) in 0.15 M NaCl. A volume of 2.5 ml of partially purified anti-CA antibodies (4 mg/ml) was added to 2.5 ml of activated Sepharose beads in a screw-capped test tube and incubated by rocking the tube at 50 cycles per min at 4°C for 12 h. Coupling of the partially purified antibody to the Sepharose beads was determined by testing for anti-CA agglutinins remaining in the supernatant after incubation. To determine if the anti-CA-coupled beads would bind candidal antigen, ^{125}I [PE]

was added to 0.1 ml of packed beads, incubated overnight at 4°C, and washed with PBS, and the percent binding of labeled PE was calculated. As controls the percent binding of labeled PE was determined on beads coupled with normal mouse serum and on beads coupled with normal rabbit serum. The antibody-Sepharose conjugate (anti-CA-Sepharose) was washed with PBS, resuspended in PBS + 0.02% NaN₃, and stored at 4°C. To saturate nonspecific binding sites, anti-CA-Sepharose was adsorbed with normal mouse serum prior to use in the RIA.

Infection of mice and postinfection processing.

C. albicans 9938 was grown under constant aeration in glucose-yeast extract-peptone broth for 25 h as described above and washed three times in 0.15 M NaCl (saline). Over 99% of the cells were in the yeast phase and were viable as determined by plating onto Sabouraud-dextrose agar. Male or female BALB/c mice, 8 to 15 weeks old, were injected intravenously with 2×10^6 yeast cells in 0.1 ml of saline and processed on day 3 or day 5 postinjection as described below. At the same time, other BALB/c mice were injected intravenously with 4×10^6 cells in 0.1 ml saline and processed on day 1 or day 3 postinjection. Both yeast cell concentrations are near the 100% lethal dose for mice receiving this strain of *C. albicans* intravenously.

Processing consisted of bleeding the infected mice by orbital venous plexus puncture, allowing the blood to clot overnight at 4°C, and centrifuging ($800 \times g$, 20 min) to obtain the serum. These sera were either assayed individually or pooled prior to use in the RIA. The clotted portion of individual or pooled blood was homogenized in sterile saline and plated onto Mycosel agar (BBL, Cockeysville, Md.). The colony-forming units of *C. albicans* that developed after approximately 36 h of incubation at 37°C in a humidified incubator were counted. Kidneys were removed from all mice, treated individually or pooled into the same groups as blood pools, and homogenized in sterile saline with a glass tissue homogenizer. Dilutions of the kidney homogenates were plated onto Sabouraud-dextrose agar, and colony-forming units of *C. albicans* were again determined. Uninfected BALB/c mice used as negative controls were processed in the same manner.

RIA procedure. Pooled serum samples from either infected mice or uninfected control mice were divided into two equal parts and assayed in duplicate. Because of the small blood volume obtained from individual mice, individual sera were not assayed in duplicate. A packed volume (0.05 ml) of anti-CA-Sepharose beads was added to these mouse sera in disposable polystyrene tubes (12 by 75 mm; Falcon Plastics, Oxnard, Calif.). Sodium azide was added to each tube to a final concentration of 0.02%. The serum samples containing the anti-CA-Sepharose were incubated for 12 h at 22 to 24°C and rotated at 150 rpm. After incubation the beads were washed twice with PBS (containing 0.02% NaN₃), with care taken not to lose beads during the washing process. The beads were suspended in 0.2 ml of PBS containing 1% bovine serum albumin and 0.02% NaN₃. Ten microliters of ¹²⁵I-labeled anti-CA antibodies (specific activity 0.22 mCi/mg) was added to the tubes and incubated for 12 h as described above. After incubation, the tubes were counted in the Beckman

Biogamma (total count); the beads were washed twice with PBS and counted again (washed bead count); and supernatant liquids obtained from the washings were also counted. Percent binding of ¹²⁵I-anti-CA to the antigen-anti-CA-Sepharose complex was determined by dividing the washed count by the total count and multiplying by 100. The percent binding obtained using serum from *C. albicans*-infected mice was compared with the percent binding of ¹²⁵I-anti-CA to anti-CA-Sepharose obtained using serum from uninfected mice.

RESULTS

Purification of antibody. Anti-CA agglutinin titers determined on unfractionated immune serum, immune serum that had been passed through the PE-affinity column, monosaccharide-eluted material, and immune serum washed through a negative control column showed that anti-CA would bind and could be eluted from the affinity column (Table 1). Glucose and mannose eluted at least 50% of anti-CA antibody activity from the column. Although at least 50% of the agglutinin activity was recoverable from the affinity column, less than 10% of the original protein content of the immune serum was found in the active eluates (Table 2).

The partially purified antibody preparation had the same degree of cross-reactivity against species other than *C. albicans* as unfractionated immune serum. Agglutinin titers were identical against both strains of *C. albicans*, but were eightfold less against *C. tropicalis* and *S. cerevisiae*. Agglutination did not occur when species of *Cryptococcus* were used as antigens. Inhibi-

TABLE 1. Anti-CA agglutinin activity of whole and fractionated immune serum

Treatment of immune serum	Agglutinin activity (%)
None	100.0 ^a
Washed twice through PE-affinity column	4.7 ^b
0.5 M glucose eluate from PE-affinity column, reconcentrated to volume added to column	50.0
0.5 M mannose eluate from PE-affinity column, reconcentrated to volume added to column	25.0
Pooled glucose-mannose eluates, reconcentrated to original volume	50.0
Washed through negative control column ^c	100.0 ^b

^a Agglutinin titer of unfractionated immune serum was 128.

^b Agglutinin activities of washes were corrected for the increase in volume that occurred during passage through the column.

^c Negative control beads were prepared by incubating activated Sepharose beads + buffer without PE, then treating with beta-mercaptoethanol.

tion of agglutinin activity of partially purified anti-CA antibodies was dependent upon the concentration of monosaccharides added to the diluent (PBS). Although the 0.5 M mixture caused significant reduction in agglutination activity, 0.125 M or less did not seem to have an effect (Table 3).

Activity of the radiolabeled partially purified antibody (^{125}I -anti-CA) preparation. By precipitating a portion of the ^{125}I -anti-CA preparation with 5% trichloroacetic acid (23) and determining the number of counts per minute that were retained in the washed pellet, it was found that 95% of the counts were in the trichloroacetic acid precipitate. To determine if the ^{125}I -anti-CA preparation had binding activity, a portion of the preparation was diluted 1:50 in PBS and absorbed $3\times$ with 1/10 volume of packed *C. albicans* yeast cells. It was found that 63% of the labeled antibody was bound to the cells. When a solution containing ^{125}I was absorbed with *C. albicans*, less than 1% of the label was associated with cells.

Activity of anti-CA-Sepharose beads. After incubating the partially purified antibody with cyanogen bromide-activated Sepharose beads and removing the beads, the agglutinin titer of the remaining supernatant liquid was 2, as compared to 64 before incubation. It was determined that antibody attached to the Sepharose would still bind antigen because, after incubation of anti-CA-Sepharose with ^{125}I [PE], 54% of the counts were retained by the beads, whereas normal mouse serum-coupled beads retained 10% of the ^{125}I [PE] and normal rabbit serum-coupled beads retained 5.5% of the ^{125}I [PE]. When uncoupled ^{125}I was added to the anti-CA-Sepharose, less than 1% of the counts remained on the beads.

TABLE 2. Protein content of various antibody preparations

Antibody preparation	Protein content (mg/ml) ^a
Unfractionated immune serum	55.7
Pooled 0.5 M glucose-mannose eluate (partially purified antibody) ^b	4.1
Partially purified antibody absorbed with <i>C. albicans</i> ^c	0.78

^a Protein determinations by the method of Lowry et al. (13).

^b Restored to volume of the original immune serum that was washed through the PE-affinity column, and dialyzed against PBS.

^c Absorbed $3\times$ with 1/10 volume of packed *C. albicans* cells; protein determination was made on the supernatant liquid.

TABLE 3. Inhibitory activity of monosaccharides on agglutinin activity of partially purified anti-CA antibodies

Glucose + mannose concn added to PBS ^a	Agglutination titer
None	64
0.5 M	8
0.25 M	16
0.125 M	64
0.06 M	64

^a Used as the diluent in the determination of agglutination titer.

Sensitivity of RIA. Using the RIA procedure as described in Materials and Methods, the minimum amount of candidal antigen that could be detected by the test was found by adding varying amounts of PE to normal mouse serum and anti-CA-Sepharose and determining the percent binding of ^{125}I -anti-CA. If PE was not added to the mouse serum, about 4.3% of the counts were associated with beads (nonspecific binding). Data obtained from these experiments indicate that a positive test occurs if at least 100 ng of *C. albicans* antigen is present in the serum sample (Fig. 1).

RIA on serum from *C. albicans*-infected mice. In all groups of infected animals assayed at various times, the percent binding of ^{125}I -anti-CA to the solid phase was greater than binding that occurred in sera from uninfected animals (Fig. 2; Table 4). Although in many instances a few organisms could be cultured from blood of infected mice, by day 5 only 10% of the blood samples yielded viable *C. albicans*, yet 70% were positive in the RIA, indicating antigenemia. The kidneys from all infected animals were positive for viable *C. albicans* (Table 4). In this RIA test, the greatest experimental error for individual sera from uninfected animals was 27%; thus binding values above 7.4% are considered indicative of candidal antigenemia. Accordingly, there were 8/27 or 29.6% false negatives (Fig. 2).

DISCUSSION

Using an RIA procedure developed in our laboratory to detect candidal antigens in mouse serum, we were able to distinguish most *C. albicans*-infected animals from uninfected controls. The development of the RIA procedure included partially purifying, by affinity chromatography, *C. albicans*-specific antibodies.

Partial purification of antibodies was achieved through the use of a *C. albicans* cell wall antigen-coupled affinity column. Over 95% of the agglutinin activity was removed from immune serum after passage through the column, and at

FIG. 1. Binding activity of ^{125}I -anti-CA to the PE-anti-CA-Sepharose complex. Anti-CA-Sepharose was incubated in normal mouse serum containing various amounts of PE, washed, then incubated in PBS containing ^{125}I -anti-CA.

least 50% of the agglutinin activity of immune serum was recovered by eluting the column with glucose and mannose and dialyzing away the monosaccharides. Incomplete recovery of agglutinin activity was probably due to retention of antibody by the column. In a pilot experiment an additional wash of the column with 1.0 M glucose-mannose eluted an additional 12% agglutinin activity. The single-step fractionation procedure reduced the amount of starting protein in the immune serum from over 50 mg/ml to about 4 mg/ml. Most of the protein in the final solution (4 mg/ml) appeared to have antibody-binding activity, because 81% of the protein adsorbed to *C. albicans* yeast cells. Our results also indicated that antigen-binding activity of antibody was retained either after coupling antibody to Sepharose beads (anti-CA-Sepharose conjugate) or radioiodinating the antibody preparation (^{125}I -anti-CA).

Although the mechanism of elution from the affinity column is unknown at this time, it is possible that glucose and mannose haptenically elute the antibodies. Because glucose is present in serum we examined the effects of various

concentrations of glucose and mannose on the agglutinin activity of the partially purified antibody preparation. Although high concentrations (0.5 M) of the monosaccharides partially inhibited agglutinin activity, 0.125 M or less of

FIG. 2. Distribution of RIA results of sera of uninfected and *C. albicans*-infected mice. Results are expressed as percent binding of ^{125}I -anti-CA to anti-CA-Sepharose in five different groups of animals: A, uninfected; B, infected with 2×10^6 *C. albicans*, assayed on day 3 postinjection; C, injected with 2×10^6 *C. albicans*, assayed on day 5; D, injected with 4×10^6 *C. albicans*, assayed on day 1; E, injected with 4×10^6 *C. albicans*, assayed on day 3. Experimental error for individual samples from infected animals was always less than 5%. The error for samples from control mice was less than 10%, with the exception of four binding values (3.7, 4.3, 4.4, and 4.6), which varied from 10 to 27%.

TABLE 4. RIA test results and recovery of viable *C. albicans* from infected and uninfected mice

Intravenous infection with <i>C. albicans</i>	Time assayed postinfection	RIA mean % binding $\pm$ SD ^a	Mean (range) of CFU ^b /g of kidney ($\times 10^6$)	Mean (range) of CFU per 0.5 ml of blood
Uninfected		4.7 (0.7)	0	0
2×10^6	3 days	8.1 (1.6)	11.3 (0.9-44)	7.5 (0-24)
2×10^6	5 days	7.8 (1.8)	7.5 (0.9-26)	0.2 (0-2)
4×10^6	24 h	8.6 (0.11)	3.8 (1.8-5.0)	6.0 (4-8)
4×10^6	3 days	10.2 (1.20)	22.3 (13-41)	5.3 (0-12)

^a SD, Standard deviation of the mean of at least six values.

^b CFU, Colony-forming units of *C. albicans*.

glucose and mannose had no detectable effect. Considering the normal range of glucose in human serum (70 to 110 mg/100 ml), this value would be far less than a concentration required for inhibiting antibody activity.

The candidal antigen used in preparing the affinity column was an aqueous-phase hot-phenol extract of whole cells. This material is identical to that prepared previously (4) and is chemically similar to cell wall constituents. Antigen obtained from different batches of phenol-extracted cells reacted similarly against rabbit anti-whole cell *C. albicans* serum (our unpublished data); therefore it appears that this extraction procedure is suitable for obtaining a cell wall component of consistent antigenic composition. Because the column effectively removed antibodies in immune serum produced by rabbits in response to whole cells of *C. albicans*, it might be expected that this polysaccharide antigen is released into the circulation upon lysis or disruption of the fungal cells in an infected animal. In fact this may occur in human disease, since other workers have successfully demonstrated cell wall-like antigenemia in human cases of systemic or mucocutaneous candidiasis (1, 15, 21).

In our experiments the anti-CA-Sepharose conjugate, used as the solid phase, was added to sera from *C. albicans*-infected mice and uninfected controls. After an appropriate incubation to allow adsorption of candidal antigen to the solid phase, ^{125}I -anti-CA was used to develop the results. In 19 out of 27 sera from infected animals, the binding ^{125}I -anti-CA was greater than in control sera. Depending upon the amount of serum obtained after bleeding infected animals, the volume of serum used in the RIA procedure varied from 0.04 ml (from which a positive test occurred) to 0.2 ml.

The amount of nonspecific binding obtained using sera from uninfected mice was constant over the range of 0.07 to 0.2 ml. Systemic disease in all of the infected mice was confirmed by demonstrating the presence of viable *C. albicans* in the kidneys. Therefore, although the sensitivity of the RIA procedure described here indicated that a serum specimen should contain at least 100 ng of antigen, positive indications of antigenemia were obtainable. Because extractable cell wall antigens of *C. albicans* are chemically similar to other yeasts such as *S. cerevisiae* (18), the finding of cross-reacting antibodies in our system is not surprising. Using antibodies prepared as reported here, the RIA test might be expected to detect systemic candidiasis due to species of *Candida* other than *C. albicans*. Because chemotherapy of candidiasis is identical

regardless of the species, cross-reacting antibodies should be viewed as a favorable condition.

There are several advantages of the RIA procedure over other methods in the detection of systemic candidiasis. Culturing for viable *C. albicans* is time consuming, which may cause delay in initiating anticandidal chemotherapy (10), and large numbers of false negatives occur (10, 16). In the RIA procedure, results may be obtained in 26 h, and in our experiments a positive RIA result (indicating antigenemia) occurred in 12 assays of sera from which viable *C. albicans* could not be recovered in the blood. Whereas false positives often occur when assaying sera of infected patients for agglutinins and precipitins (7, 12), detection of antigen by the RIA procedure is not dependent upon an immune response by the host and should be independent of previous exposure to *C. albicans*. Clearly, because this work was done entirely on mice under controlled conditions, the procedure should be extended to trials on sera from human cases of systemic candidiasis and paired controls.

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