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# Evidence that Mannans of *Candida albicans* Are Responsible for Adherence of Yeast Forms to Spleen and Lymph Node Tissue

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**We have described a unique binding system between *Candida albicans* yeast-form cells and the marginal zone of mouse spleen (16). The chemical nature of the fungal adhesin(s) involved in this binding phenomenon was examined. A fraction obtained by 2-mercaptoethanol extraction (2-ME extract) of fungal cells caused a dose-response inhibition of yeast cell adherence to splenic marginal zone sites and also to subcapsular and medullary sinuses of mouse popliteal lymph nodes. Latex beads coated with the 2-ME extract showed a pattern of spleen and lymph node tissue binding identical to that observed with yeast cells. The extracted adhesins retained their binding activity in vivo. When 0.5 mg of the 2-ME extract was given intravenously to mice, spleen tissue removed up to 3 h later showed over 80% inhibition of yeast cell binding to the spleen marginal zone, and over 50% inhibition was retained for at least 24 h. The adhesins bound to a concanavalin A affinity column and were eluted by 0.5 M α-methyl-D-mannopyranoside, and the eluted adhesins were designated Fr.II. Fr.II was further fractionated by DEAE-Sephacel ion-exchange column chromatography, and one especially active and abundant fraction was designated Fr.IIa. The adhesin moiety appeared to be carbohydrate, because the activity of Fr.IIa was destroyed by 20 mM sodium periodate or by 5 U of α-mannosidase, but boiling (30 min) or proteinase K (100 μg/ml) treatments had no effect. Chemically, whereas the 2-ME extract contained significant amounts of protein and mannose, Fr.IIa consisted of over 98% mannose and less than 0.5% protein. These data strongly suggest that the mannan portion within a mannoprotein is responsible for the binding of yeast cells to splenic marginal zone and to subcapsular and medullary sinuses of mouse lymph node tissue.**

A variety of characteristics of *Candida albicans* have been associated with virulence of this organism (3, 5, 8, 12, 27). Surface molecules (adhesins) of the fungus give the fungus its adherence properties and are likely to be important in initiation of host-*C. albicans* interactions. Cell wall mannoproteins are responsible for adherence properties of *C. albicans*, although some studies show a role for cell wall lipids (9), and chitin is an important consideration in attachment of *C. albicans* to the vaginal epithelium (22). The protein portion of mannoproteins appears to contain an adhesin site for yeast cell attachment to buccal epithelial cells (31) and to endothelial cells and arginine-glycine-aspartic acid (RGD)-containing host glycoproteins (11, 19) and is at least partially responsible for hydrophobic properties of yeast cells (14). Yeast cell attachment to the buccal epithelium and the receptor for fibrinogen may also involve determinants within the mannan portion of mannoproteins (2, 26).

Adhesins of *C. albicans* yeast cells most likely determine which host tissues the fungus will seed as a consequence of candidemia. An integrin-like molecule on the surface of yeast and especially hyphal forms of the fungus may be responsible for at least some of the interactions of *C. albicans* with host tissues (11, 32). Whether a single or multiple molecules of the fungus are involved in interactions with endothelial cells and RGD-containing host glycoproteins (as cited above) is not known. However, RGD peptides have been shown to influence the ability of the host to handle *C. albicans* (20, 29), and a theory of the pathogenesis of disseminated candidiasis that implicates a critical interaction of fungal cell wall protein(s) with host endothelial cell and extracellular matrix proteins has been proposed (19).

Recently, we reported on adherence characteristics of *C. albicans* yeast cells to splenic (6, 13, 16, 28) and lymph node tissues (6). We found that when *C. albicans* is grown at 37°C, yeast cells adhere preferentially to marginal zone macrophages within the spleen (16). This binding activity was demonstrated by using an ex vivo binding assay adapted from studies on leukocyte homing receptors (1, 15, 30). Furthermore, when yeast cells are used to induce candidemia in mice, the fungal cells adhere to marginal zones of the spleen in a pattern essentially identical to that observed with the ex vivo binding assay (6). Evidence that marginal zone macrophages of mice express a binding adhesion system not found on macrophages in other regions of the spleen or in other tissues such as the thymus was presented.

In this report we provide evidence that mannans, and not proteins, expressed by *C. albicans* yeast cells are responsible for adherence of the fungal cells to mouse spleen and lymph node tissue. When isolated from *C. albicans* yeast cells, these adhesins retain their ability to bind to specific tissue locations in the spleen and lymph node, as evidenced by the ex vivo binding assay and by in vivo experiments.

## MATERIALS AND METHODS

**Organisms and culture conditions.** *C. albicans* A9 (serotype B) was used for most of the studies. *C. albicans* 1 (serotype A) and 222 (serotype B) were also used as indicated. All strains were previously described (6, 17, 23). Cultures were started each week from frozen glycerol stocks as previously described (16) and appropriately transferred to

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glucose-yeast extract-peptone (GYEP) to ensure that the yeast-form cells were in stationary phase and had a hydrophilic surface (13, 16).

**Extraction and fractionation of the mannan adhesins.** Yeast cells were grown in GYEP as described above at 37°C. The stationary-phase cells were washed in deionized water and then in 0.1 M disodium EDTA, pH 7.5. The centrifuged pellet of cells was suspended and extracted in 0.3 M 2-mercaptoethanol (2-ME) in 0.1 M trisodium EDTA, pH 9.0, for 30 min at 21 to 23°C with occasional mixing. The extract was dialyzed against deionized water at 5 to 8°C until 2-ME odor could no longer be detected. The deionized water was then changed one more time, dialysis was allowed to proceed for another 4 to 6 h, and the material was lyophilized and referred to as the 2-ME extract.

The 2-ME extract was further fractionated by affinity chromatography on a concanavalin A (con A)-agarose column. Two hundred milligrams of 2-ME extract was dissolved in 3 ml of pH 7.2 phosphate buffer containing 0.15 N NaCl (PBS) and applied to a con A-agarose (Honen Co., Tokyo, Japan) column (20 by 120 mm). After application of the sample, the column was washed (20 ml/h) with 200 ml of PBS until the $A_{220}$ and $A_{280}$ of the eluate fractions (5 ml/fraction) were negligible. The total PBS wash was pooled and referred to as Fr.I. Material that bound to the column was eluted with 0.5 M α-methyl-D-mannopyranoside (Wako Pure Chemicals, Osaka, Japan) under the same conditions as PBS elution, and the 5 ml fractions were monitored by reading $A_{220}$ and $A_{280}$. The methyl mannopyranoside-eluted fractions were pooled and referred to as Fr.II. Fr.I and Fr.II were dialyzed against deionized water at 5 to 8°C, lyophilized, and tested for the presence of adhesins as indicated below.

Fr.II contained essentially all of the adhesin activity and was further fractionated by ion-exchange chromatography. Fr.II (80 mg) was dialyzed against 20 mM Tris-HCl buffer (pH 7.8) overnight at 5 to 8°C. The dialyzed sample was applied to a DEAE-Sephacel (Pharmacia-LKB Biotechnology) column (20 by 100 mm) and washed with 200 ml of 20 mM Tris-HCl buffer. Material was eluted with a linear gradient of 0 to 0.3 M NaCl made in 20 mM phosphate buffer (total 500 ml) (pH 7.8) at a flow rate of 40 ml/h. The fractions were monitored for $A_{220}$ and $A_{280}$. All eluted materials were divided into three pools on the basis of absorbancy patterns and referred to as Fr.IIa, Fr.IIb, and Fr.IIc. The three fractions were dialyzed against deionized water and lyophilized. All fractions were monitored by sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis (PAGE) (see below) and also tested for adhesin activity by measuring their ability to inhibit attachment of yeast cells to the marginal zone of splenic tissue in the ex vivo assay as indicated below.

**SDS-PAGE.** SDS-PAGE was used to monitor adhesin fractionation. Procedures followed were similar to those described by Laemmli and Favre (21). Sample loading was 10 μl in all cases, and the concentration of each sample was standardized to 1 mg of carbohydrate per ml of sample. After fixation, the 10% polyacrylamide gels were stained with Coomassie brilliant blue R for protein or were treated with periodic acid-Schiff reagent (18) to stain for polysaccharides.

In some cases, SDS-polyacrylamide gels were electroblotted onto nitrocellulose sheets [Cellulosenitrat(E) BA 85; Schleicher & Schuell, Dassel, Germany] for 3 h at 100 V. After transfer, the nitrocellulose was blocked in 20 mM PBS containing 3% bovine serum albumin (BSA) for 30 min at 21 to 23°C. The nitrocellulose was incubated for 60 min with an overlay of con A-horseradish peroxidase (Vector Laboratories, Inc., Burlingame, Calif.) diluted 1:100 in 1% BSA-PBS, washed three times in 1% BSA-PBS, soaked in 20 mM Tris-HCl plus 0.5 N NaCl buffer (pH 7.8) (TBS), and incubated for 15 min at 21 to 23°C in a mixture of 50 ml of TBS plus 30 μl of 30% $H_2O_2$ plus 10 ml of ethanol containing 30 mg of 4-chloro-1-naphthol for 15 min at 21 to 23°C.

**Physical, chemical, and enzymatic treatments of the adhesin fraction.** Fr.IIa from the DEAE-Sephacel column was dissolved in deionized water at 1 mg/ml; boiled for 5, 15, or 30 min; diluted 1:100 in Dulbecco's modified Eagle's medium (DMEM; pH 7.4; Sigma) and tested for adhesin activity in the ex vivo assay. Fr.IIa (1 mg/ml) was incubated in 5, 10, 20, or 50 mM sodium periodate (Wako Pure Chemicals) in 50 mM acetate buffer, pH 4.5, for 30 min in the dark at 5 to 8°C. After incubation, the solution was neutralized with 1 N NaOH, dialyzed against deionized water for 24 h with water changes every 4 to 6 h, and diluted 1:100 in DMEM for the adherence assay. Fr.IIa (1 mg/ml) in 50 mM Tris-HCl buffer (pH 7.5) was incubated in the presence of 50 and 100 μg of proteinase K (E. Merck, Darmstadt, Germany) (which was preactivated by heat at 50°C for 30 min) per ml under constant reciprocal shaking for 60 min at 37°C. Protein was removed by adding an equal volume of water-saturated phenol to the mixture, vigorously shaking, centrifuging for 10 min at 3,000 × *g*, carefully removing the aqueous phase containing the adhesin, and recovering the adhesin as a precipitate which formed upon addition of 3 volumes of cold ethanol. The precipitate was dissolved in DMEM to give 10 μg of adhesin fraction per ml and tested in the adherence assay. For α-mannosidase treatment, Fr.IIa (1 mg/ml) was dissolved in 0.1 M citrate buffer (pH 5.0) containing 0.1, 1.0, or 5.0 U of α-mannosidase (Sigma) and incubated for 16 h at 25°C. After treatment, samples were boiled for 5 min to inactivate the enzyme and diluted 1:100 in DMEM.

As a control for sodium periodate treatment, the same concentration of Fr.IIa was incubated in 50 mM acetate buffer for 30 min in the dark at 5 to 8°C. As a control for α-mannosidase digestion, Fr.IIa (1 mg/ml) was dissolved in 0.1 M citrate buffer (pH 5.0) and incubated for 16 h at 25°C. Chicken egg albumin (ovalbumin) (Sigma) was used to check activity of α-mannosidase by observing a decrease in molecular weight by SDS-PAGE of treated compared with that in untreated ovalbumin. Azocoll (Sigma) was used to show that 5 U of α-mannosidase did not have proteolytic activity. Azocoll was also used to show activity of proteinase K.

**Chemical analyses.** Carbohydrate analysis in which gas capillary columns were used to analyze alditol acetate and trimethylsilyl (TMS) derivatives of the sugar components was done as previously described (33). The following standards (obtained from Sigma) were also derivatized: alditol acetate standards, including rhamnose, fucose, arabinose, xylose, galactose, glucose, and mannose; and TMS standards, including all of the alditol acetate standards and glucuronic acid, galacturonic acid, *N*-acetyl glucosamine, and *N*-acetyl galactosamine. Alditol acetate derivatives were separated on an SP 2330, 0.2-μm-film-thickness column (30 M by 0.25 mm; Supelco, Inc., Bellefonte, Pa.), and TMS derivatives were separated on a DB-1, 0.25-μm-film-thickness column (30 M by 0.25 mm; J & W Scientific, Folsom, Calif.). Both columns were run in a Varian 3700 gas chromatograph, and output was recorded (Shimadzu C-R6A Chromatopac). Protein content was determined by a commercial assay (Bio-Rad Protein Assay; Bio-Rad Laboratories, Richmond, Calif.) in which BSA (Sigma) was used as the standard.

**Adhesin coating of latex particles.** Latex beads (4.51 μm diameter, polystyrene latex, surfactant free; IDC Spheres, Portland, Oreg.) were gently suspended in the manufacturer's vial, 0.25 ml was withdrawn (containing 8.1% solids), and the beads were pelleted by centrifugation and suspended in 1 ml of pH 9.6 0.067 M carbonate buffer to give a 2% (vol/vol) suspension of beads. One milligram of 2-ME extract was suspended in 1 ml of the carbonate buffer and combined with the 1 ml of the 2% suspension of latex beads, and the mixture was allowed to mix at 21 to 23°C for 16 to 18 h under constant rotation. BSA-coated control beads were prepared by making a suspension of beads in PBS and adding an equal volume of 1% BSA in PBS and mixing for 1 h at 21 to 23°C. Both preparations of beads were washed several times in PBS containing 1% BSA and stored at 5 to 8°C until use. Evidence that latex beads became coated with 2-ME extract was obtained by observing agglutination of the beads in the presence of rabbit polyclonal antiserum raised against whole cells of *C. albicans*, whereas BSA-coated beads did not agglutinate.

**Ex vivo adherence assay.** The stationary method (16, 28) was used in all studies on spleens and lymph nodes with minor modifications as indicated. The tissues were obtained from male or female mice that were between 7 and 16 weeks old and were either BALB/cByJ, BALB/cByA, or [BALB/cByJ × Crl:CD-1(ICR)BR]F1 strains. Spleens were obtained 5 min after intravenous (i.v.) inoculation of 0.1 ml of 10% luconyl blue (44 E 3172; BASF Aktiengesellschaft). Popliteal lymph nodes were removed from animals not treated with luconyl blue. Both tissue types were placed in Tissue Tek O.C.T. compound (Miles Inc., Elkart, Ind.) immediately after removal from the animals, rapidly frozen on dry ice, and stored at −80°C until cryosectioned. Spleen and lymph node binding assays, which included cryosectioning, addition of yeast cells, incubation conditions, and subsequent tissue fixation and staining, were done exactly as described previously for splenic tissue (16). Yeast cell binding to splenic tissue was quantified by manual counting as described before (28). In all cases, at least three slides per treatment were counted, and each slide contained four tissue sections. Means and standard errors were calculated.

Binding of yeast cells to lymph node tissue was determined by computer image analysis as previously described (28) except that the M1 update of the MCID image analysis program was used (Imaging Research, St. Catherines, Ontario, Canada). Each lymph node tissue was examined with a 10× objective lens on a bright-field microscope, and the image was digitized. The number of yeast cells that bound were determined in 30 contiguous rectangular fields, 20 by 200 μm, from the subcapsular and medullary regions of the lymph node. Consistent with previous observations (6), these are the regions which showed the greatest number of yeast cells binding to lymph node tissue. Measurements were made from 12 lymph node tissues for each treatment, and results were expressed as the estimated mean number of adherent yeast cells per rectangular field. Standard errors of the means were also calculated.

Binding of latex beads coated with the 2-ME extract to splenic tissue was done exactly as defined above for binding of yeast cells to the spleen tissue, except that 100 μl of the beads ($1.5 \times 10^8$/ml) was allowed to interact with the splenic tissue for 45 instead of 15 min as described for the yeast cells.

**Test for solubilized adhesins by the ex vivo assay.** The soluble 2-ME extract or soluble fractions of the extract diluted in DMEM, as indicated above, were tested for adhesin activity by pretreating frozen sections of tissue with these substances before application of yeast cells in the ex vivo assay. If adhesins were present in the solubilized materials, pretreatment of the tissues should block the attachment of yeast cells. For these pretreatment experiments, 100-μl amounts of each fraction or extract in DMEM (buffered to pH 7.4 with 25 mM *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid [HEPES]) (Sigma) plus 5% fetal bovine serum were added to the tissue sections at 5 to 8°C. Tissues were placed on a rotator for the first 5 min of the 15-min incubation period at 5 to 8°C. The 100 μl of test material was then decanted, the slide edge was blotted, and the yeast cells suspended in DMEM were added and allowed to interact with the tissue for 15 min at 5 to 8°C without rotation. As a control for the pretreatment, some tissue sections were pretreated with DMEM (buffered with HEPES as described above) alone prior to addition of yeast cells.

**In vivo treatment with 2-ME extract.** Mice were given the 2-ME extracted adhesins i.v. prior to removal of the spleen for subsequent ex vivo binding assays. The animals were given 0.1 ml of the 2-ME extract at various concentrations, 60 min later the animals were given 0.1 ml of 20% luconyl blue, and 5 min later they were sacrificed by cervical dislocation and their spleens were frozen in O.C.T. as described above and stored at −80°C until sectioning and use in the ex vivo assay. In other experiments, animals were given i.v. 0.1 ml of the 2-ME extract at 5 mg of PBS per ml. At 15 and 30 min and 1, 3, 6, or 24 h after injection, mice were given 0.1 ml of 20% luconyl blue, and 5 min later they were sacrificed and their spleens were prepared for the ex vivo assay as described above. Spleens from animals given 0.1 ml of PBS i.v. instead of the 2-ME extract were used as positive controls for yeast cell binding.

## RESULTS

**Evidence that the 2-ME extract contains the *C. albicans* adhesins.** The 2-ME extract caused a dose-response inhibition of yeast cell binding (strain A9) when tissues were pretreated with various doses of the extract (Fig. 1). A slight inhibition of binding of yeast cells was noted when spleen tissue was pretreated with 0.1 μg of the extract per ml (i.e., 0.01 μg of extract was actually applied to the tissue). There was no indication that the adhesins liberated during the extraction were strain specific, because the 2-ME extract from strain A9 caused an almost identical dose-response inhibition of marginal zone binding of strains 1 and 222. Also, 2-ME extract prepared from strain 1 caused a similar dose-response inhibition of binding of strains 1 and A9 (data not shown).

Inhibition of binding of yeast cells to the subcapsular and medullary sinuses of lymph node tissue also occurred in a dose-response fashion essentially identical to that of splenic tissue (not shown). Latex beads coated with the 2-ME extract bound to the marginal zone of the spleen (Fig. 2A) and to the subcapsular and medullary sinuses of the lymph node (Fig. 2B). Latex beads coated with BSA did not exhibit this binding pattern (not shown). Furthermore, if the tissues were pretreated with soluble 2-ME extract (10 μg/ml), binding of the 2-ME-coated latex beads was inhibited.

The 2-ME extract was also active in vivo. Spleens obtained 1 h after mice were given the 2-ME extract i.v. had reduced yeast cell binding (Fig. 3). The amount of inhibition was dependent on the dose of 2-ME extract administered. The amount of inhibition of yeast cell binding to the marginal zone of the spleen remained over 80% for up to 3 h following

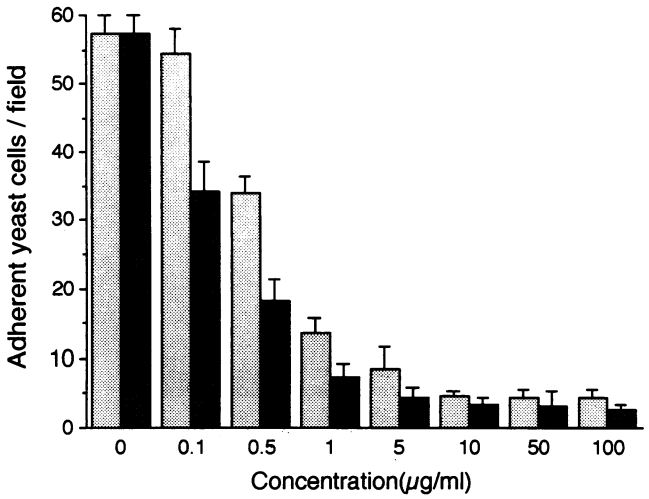


FIG. 1. Inhibitory effects of 2-ME extract and Fr.IIa on binding of *C. albicans* to spleen marginal zone in the ex vivo binding assay. Splenic tissue was pretreated with various concentrations of the 2-ME extract prior to addition of yeast cells to the tissue. The amount of yeast cell binding was compared with that in tissue treated with medium alone (column 0). Dotted columns, 2-ME extract; lined columns, Fr.IIa. Standard error bars were calculated for each experimental condition.

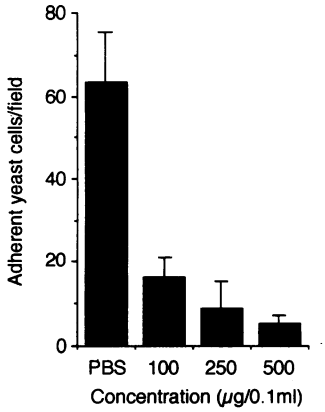


FIG. 3. Adhesin activity of the 2-ME extract in vivo. Mice were injected with various doses of 2-ME extract in PBS i.v., and their spleens were removed 1 h later and used in the ex vivo binding assay. A dose-response inhibition of binding was obtained compared with that in control animals given PBS only. Standard error bars were calculated for each experimental condition.

an i.v. injection of 500 μg of the extract, and over 50% inhibition was still evident 24 h later (Table 1).

**Fractionation of the 2-ME extract.** Subsequent fractionation of the 2-ME extract by con A-agarose chromatography yielded one fraction that did not bind to the column (Fr.I) and one fraction that was eluted with α-methyl-D-mannopyranoside (Fr.II). The majority of adhesin activity was associated with Fr.II (see Fig. 5, below). Fraction II was further chromatographed on a DEAE-Sephacel column, and a total of three peak areas, designated Fr.IIa, Fr.IIb, and Fr.IIc, were eluted during washing of the column with a 0 to 0.3 N NaCl linear gradient. Evidence of separation was obtained by monitoring each fraction by SDS-PAGE (Fig. 4). With each fractionation step, the amount of protein as detectable by Coomassie blue staining decreased (Fig. 4A) while the amount of con A-reactive material tended to increase (Fig. 4B). The respective carbohydrate and protein determinations for each fraction (on a microgram per milligram [dry weight] basis) were 2-ME extract, 850 and 75; Fr.I, 220 and 680; Fr.II, 920 and 38; Fr.IIa, 970 and 35; Fr.IIb, 975 and 33; and Fr.IIc, 960 and 25.

The various fractions of the extract were tested for their adhesin activities by pretreating tissue sections and determining the pretreatment effect on yeast cell binding. When each fraction was tested at 50 μg/ml, the adhesin activity was found to be especially strong in Fr.IIa and Fr.IIb (Fig. 5). Fr.IIa gave the highest yield of material and was chosen for further studies.

The 2-ME extract and Fr.IIa were compared for their

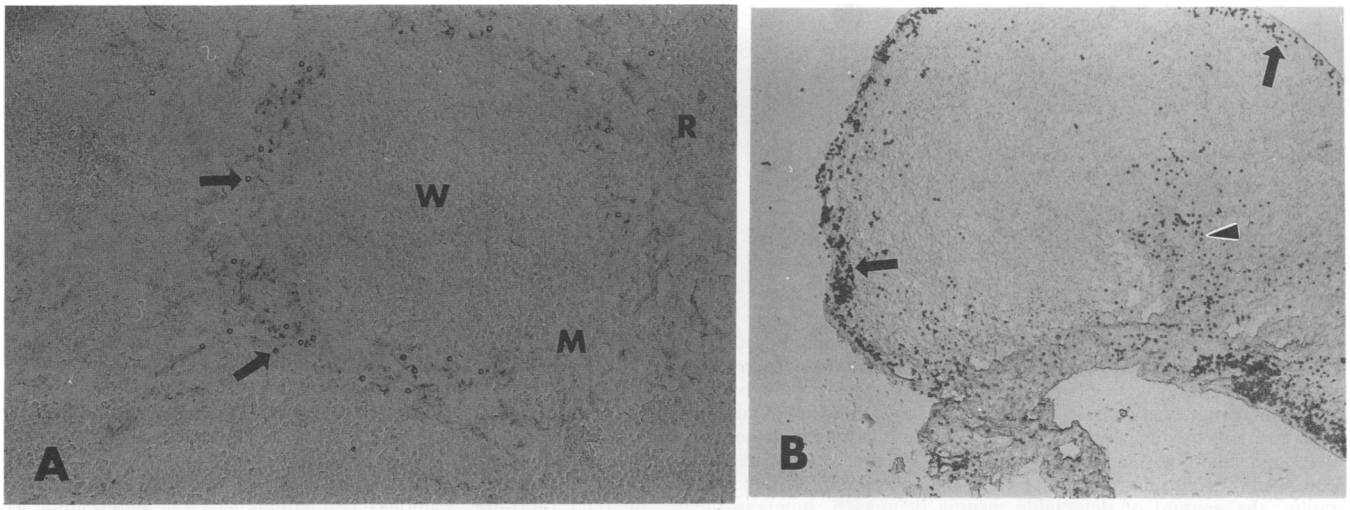


FIG. 2. Adherence of 2-ME latex-coated beads to spleen and lymph node tissue. Latex beads were coated with 2-ME extract or with BSA as a control and used in the ex vivo assay. Beads coated with 2-ME extract adhered to the marginal zone of the spleen (A, arrows) and to the subcapsular (arrows) and medullary sinuses (arrow head) of lymph node tissue (B). BSA-coated latex beads did not bind to these areas (not shown). W, white pulp; R, red pulp; M, marginal zone. Average diameter of latex beads, 4.51 μm.

TABLE 1. Effects of i.v. treatment of mice (in vivo) with 2-ME extract on ex vivo binding of *C. albicans* yeast cells to the splenic marginal zone

| Treatment time[a] (h) | Adherent yeast cells/ field (SE) | % Inhibition |
|---|---|---|
| 0.25 | 6.8 (5.1) | 90.4 |
| 0.5 | 7.5 (4.0) | 88.9 |
| 1 | 8.8 (3.9) | 86.9 |
| 3 | 10.5 (6.5) | 84.4 |
| 6 | 27.3 (6.8) | 59.5 |
| 24 | 30.9 (11.0) | 54.0 |
| 24[b] | 67.1 (11.7) | |

[a] Mice were given 0.1 ml containing 5 mg of 2-ME extract per ml in PBS i.v. Animals were sacrificed at various times following the pretreatment, and binding of *C. albicans* to spleen tissue in the ex vivo assay was assessed.

[b] Control mice were given 0.1 ml of PBS i.v., and their spleens were removed 24 h later and used in the ex vivo binding assay to represent 100% binding.

relative abilities to inhibit yeast cell binding to the marginal zone of the spleen (Fig. 1). Fr.IIa was slightly more active than the 2-ME extract. Fr.IIa also caused a dose-response reduction of yeast cell binding to lymph node tissue that was pretreated with various amounts of the fraction (Fig. 6). Yeast cell binding to the medullary and subcapsular sinuses of lymph node tissue was markedly reduced when the tissue was pretreated with as little as 0.1 μg of Fr.IIa per ml.

**Physical-chemical characteristics of the adhesins.** The effects of heat, periodate oxidation, and enzymatic digestions with α-mannosidase and proteinase K on the Fr.IIa adhesin material were examined. After the various treatments, the effect of pretreating splenic tissue on yeast cell binding was determined (Table 2). Neither heat nor proteinase K digestion affected the adhesin activity of Fr.IIa, yet the relative amount of protein was reduced by about 10-fold as a result of proteinase treatment. That is, the respective concentrations of carbohydrate and protein concentrations (in micrograms per milliliter) were before proteinase digestion 970 and 35 and after proteinase digestion 750 and 3.5. Treatment with

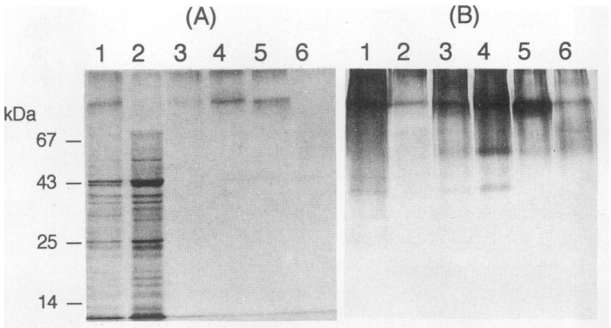


FIG. 4. Fractionation of the 2-ME extract as monitored by SDS-PAGE. The 2-ME extract and fractions obtained following various procedures were examined for the presence of protein bands by staining with Coomassie blue (A) and for mannose-containing glycoproteins by developing the gels with con A-horseradish peroxidase and substrate (B). As the 2-ME extract became fractionated by con A-affinity chromatography and DEAE ion-exchange chromatography, the amount of detectable protein decreased, whereas the amount of con A-binding material remained the same or increased. Lanes 1, 2-ME extract; lanes 2, Fr.I; lanes 3, Fr.II; lanes 4, Fr.IIa; lanes 5, Fr.IIb; lanes 6, Fr.IIc. Molecular size standards are given on the left.

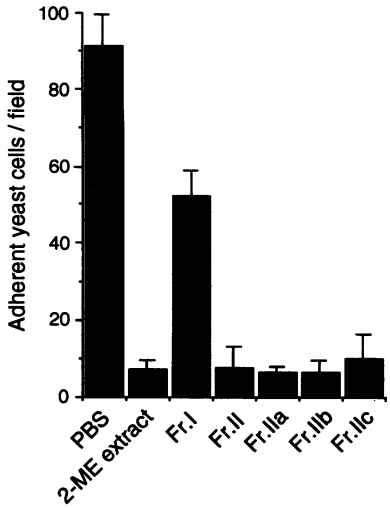


FIG. 5. Effects of adhesin fractions on ex vivo binding of *C. albicans* to the marginal zone of splenic tissue. The 2-ME extract was put onto a con A-agarose affinity column, and Fr.I and Fr.II were eluted with PBS and 0.5 M α-methyl-D-mannopyranoside, respectively. Fr.II was placed onto a DEAE-Sephacel column, and Fr.IIa, Fr.IIb, and Fr.IIc were eluted with an NaCl linear gradient (0 to 0.3 M). All fractions were dissolved in DMEM at 50 μg/ml and used to pretreat splenic tissue before addition of yeast cells. Control tissue sections were pretreated with DMEM. Except Fr.I, all fractions from the con A column showed inhibitory activity. Bars indicate standard errors.

sodium periodate at 20 mM or α-mannosidase at 5 U essentially inactivated the adhesins. The various treatments also similarly affected the in vivo activity of the 2-ME extract and Fr.IIa (Fig. 7). That is, periodate oxidation destroyed the adhesin activity (Fig. 7, treatments C and E),

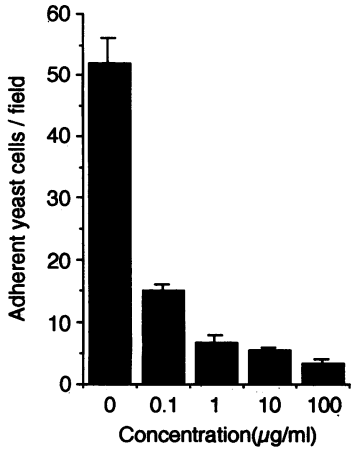


FIG. 6. Effects of Fr.IIa pretreatment of lymph node tissue on ex vivo binding of *C. albicans* yeast cells. Various concentrations of Fr.IIa were prepared, and 100 μl of each was used to pretreat lymph node tissue sections as described in Materials and Methods. One hundred microliters of yeast cells ($1.5 \times 10^8$ cells per ml) was overlaid onto the sections, and adherence was determined as described. The *y* axis shows average number of yeast cells that bound per field (bars indicate standard errors), and the *x* axis shows the concentration of Fr.IIa applied to the sections. The positive binding control received suspending medium only (0 concentration).

TABLE 2. Effects of various treatments on the ability of Fr.IIa to inhibit yeast adherence[a]

| Treatment of Fr.IIa | Adherent yeast cells/field (SE) |
|---|---|
| Heat (100°C) | |
| 5 min | 4.6 (2.7) |
| 15 min | 4.8 (1.2) |
| 30 min | 6.3 (1.8) |
| Sodium periodate (4°C, 30 min) | |
| 5 mM | 16.8 (6.1) |
| 10 mM | 32.6 (6.3) |
| 20 mM | 74.7 (5.9) |
| 50 mM | 76.9 (6.8) |
| α-Mannosidase (25°C, 16 h) | |
| 0.1 U | 8.9 (1.9) |
| 1.0 U | 32.6 (2.8) |
| 5.0 U | 68.3 (7.8) |
| Proteinase K (37°C, 60 min) | |
| 50 μg/ml | 6.7 (1.9) |
| 100 μg/ml | 7.4 (2.1) |
| No treatment | 4.6 (2.2) |
| Medium only | 78.9 (9.3) |

[a] Fr.IIa (1 mg/ml) was treated as indicated and used to pretreat splenic sections prior to addition of yeast cells in the ex vivo binding assay. Subsequent binding of yeast cells to the marginal zone was quantified as specified in Materials and Methods.

while proteinase treatment had no effect (Fig. 7, treatment F).

Chemically, when Fr.IIa TMS and alditol derivatives were compared with known sugar derivatives, the major carbohydrate component of Fr.IIa was mannose. By analysis of TMS derivatives, mannose accounted for at least 98% of the carbohydrates in the Fr.IIa, and by alditol derivative analysis, Fr.IIa consisted of slightly over 99% mannose. After proteinase K treatment of Fr.IIa, the protein content was less than 0.5%.

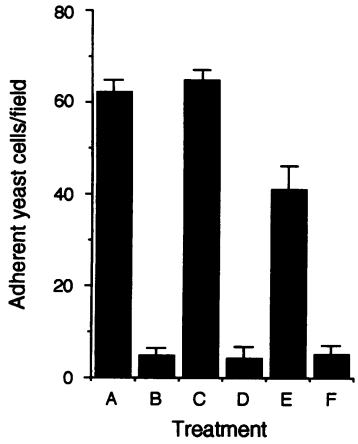


FIG. 7. Effects of sodium periodate and proteinase on in vivo blocking by 2-ME extract and Fr.II. 2-ME extract and Fr.II were treated with 25 mM $NaIO_4$ for 30 min at 4°C or proteinase K for 6 h at 37°C, and 0.1 or 0.2 ml of the treated samples was injected i.v.; 1 h after injection, spleens were obtained from the animals and used in the ex vivo assay. Spleens obtained from the animals injected with PBS (A), 2-ME extract (B), and Fr.II (D) were used as controls. Number of adherent yeast cells decreased clearly in the spleens from mice treated with the $NaIO_4$–2-ME extract (C) and $NaIO_4$-Fr.II (E). Proteinase K treatment had no effect (F).

## DISCUSSION

Recently, we reported that *C. albicans* yeast cells bind to marginal zone macrophages of mouse spleen (16). A similar adhesion system appears to be present in subcapsular and medullary sinuses of peripheral lymph node tissue (6). There is indirect evidence to suspect that yeast cell surface carbohydrates are involved in the binding. We found that the specific tissue binding characteristics pertain to hydrophilically grown yeast cells but not to hydrophobic cells (13). Hydrophobicity is due, at least in part, to a surface protein molecule (14), whereas hydrophilic cells appear to be mannosylated, as evidenced by the change from hydrophilicity to hydrophobicity when *C. albicans* yeast cells are grown in the presence of tunicamycin. Evidence presented in this paper gives further support for the mannan of *C. albicans* being responsible for the specific adherence characteristics of hydrophilic yeast cells to mouse spleen and lymph node tissues.

Evidence that the adhesins were extracted by 2-ME is strong. Others found that the cell wall mannoprotein that is responsible for fibrinogen binding to *C. albicans* yeast and hyphal forms is extractable by 2-ME (2). In our work, a 2-ME extract inhibited binding of yeast cells when tissues used in the ex vivo binding assay were pretreated prior to the addition of yeast cells. Latex beads coated with the 2-ME extract bound to splenic marginal zone cells and to the subcapsular and medullary sinuses of lymph node tissue (Fig. 2), which is the same distribution of tissue binding as whole hydrophilic yeast cells (6, 16). Also, when mice were given the 2-ME extract i.v., spleen tissue removed from these animals had a reduced ability to bind yeast cells. Although the majority of work was done on adhesins obtained from extraction of *C. albicans* A9, there was no indication that the adhesins were strain specific, because the strain A9 (serotype B) 2-ME extract also blocked attachment of the serotype A strain 1, as well as the serotype B strain 222. Also, a 2-ME extract from serotype A strain 1 blocked attachment of strain A9 to tissues.

The chemical moiety in the 2-ME extract responsible for its adherence properties appears to be from the mannan part of the molecule. Whereas the 2-ME extract contained numerous protein bands, subsequent fractionation by con A-affinity chromatography and ion-exchange chromatography removed the majority of the protein material, yet full adhesin activity remained. Boiling of the adhesin or removal of almost all of the remaining protein in the extract by proteinase K digestion did not affect the adhesin activity (Table 2). On the other hand, periodate oxidation, which may destroy oligosaccharide moieties (7, 10, 25), had a dose-response destructive effect on the adhesin (Table 2). Carbohydrates appeared necessary for adhesin activity in the ex vivo assay and in vivo, since periodate-treated adhesins lost their ability to block splenic tissue binding of *C. albicans*. In addition, intact hydrophilic yeast cells treated with periodate lost their ability to specifically bind to the splenic marginal zone and instead bound randomly to the tissues (not shown) like hydrophobic yeast cells (13). Finally, the predominant material in the adhesin fraction (Fr.IIa) after proteinase K treatment was mannose, and treatment of the Fr.IIa material with α-mannosidase virtually eliminated the adhesin activity.

The adherence of *C. albicans* to splenic tissue is not a phenomenon unique to the ex vivo assay. As reported earlier, when yeast cells are administered i.v., they rapidly become associated with the splenic marginal zone cells (6) in a tissue distribution pattern similar to that observed in the ex vivo assay. As mentioned above, the 2-ME-extracted yeast adhesins retained their binding activity in vivo, which suggests that they function in host-*C. albicans* interactions.

The 2-ME extract containing the adhesin molecules was fractionated by a similar scheme used by Douglas and coworkers on a *C. albicans* culture supernatant material (4, 31). In studies on adherence of *C. albicans* to buccal epithelial cells, they determined that the fungal adhesin molecule is a protein (31) and that expression of the molecule is *C. albicans* strain specific (24). In our work, the active adhesin moieties are due to mannan, and expression of the adhesin is not strain specific. Our experiments have also shown that the interaction of *C. albicans* yeast cells with spleen and lymph node tissue is not due to known adhesion systems (16) and indicate that the pathogenesis of disseminated candidiasis involves more than an interaction of *C. albicans* with endothelial cells or with extracellular matrix proteins.

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