Production and Characterization of Murine Monoclonal Antibodies to Histoplasma capsulatum Yeast Cell Antigens

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Four monoclonal antibodies (MAbs) were produced by immunizing mice with a disrupted yeast cell homogenate of Histoplasma capsulatum. MAbs 1 and 2 reacted only with the yeast cell antigens of H. capsulatum and Blastomyces dermatitidis, whereas MAbs 3 and 4 showed broader cross-reactivity. MAb 3 cross-reacted with B. dermatitidis, Paracoccidioides brasiliensis, Sporothrix schenckii, and Candida albicans, and MAb 4 cross-reacted with B. dermatitidis, C. albicans, Coccidioides immitis, Aspergillus fumigatus, and Mycobacterium tuberculosis. All four MAbs exhibited unique specificity when reacted with three different strains of H. capsulatum (G217B, A811, and P-IN). MAb 1 belonged to the IgG2b subclass, MAb 3 belonged to the IgG1 subclass, and MAbs 2 and 4 belonged to the IgG3 subclass. MAbs 1, 2, and 3 formed bands in the Western immunoblot assay; the two dominant distinct bands had apparent molecular masses of 72 and 62 kilodaltons.

Although serologic tests have proved to be very useful in the diagnosis of histoplasmosis, they have certain limitations. One of these limitations is false-positive results for patients with other diseases (5, 11, 12, 15, 19, 20, 27, 28, 30, 46). A number of factors could lead to such results, one of which is the presence of cross-reacting antigens in the reagents used for serodiagnosis. False-positive results have been attributed to the presence of antigens cross-reactive between Histoplasma capsulatum and other fungal pathogens (5, 27, 46). Furthermore, analysis of antigenic components of H. capsulatum and other fungal pathogens showed that the crude antigenic preparations used in fungal serodiagnosis might contain one or more common antigens (5, 11–13, 15, 16, 18–20, 23, 27, 28, 30, 32, 37, 46).

In the serodiagnosis of histoplasmosis, histoplasmin (the culture filtrate of the mycelial form of H. capsulatum) is one of the most widely used reagents. In attempts to minimize the degree of cross-reactivity, histoplasmin has been partially purified and characterized (2, 4, 22, 25, 40, 41). However, none of these studies showed that the purified reagent was specific for H. capsulatum.

H. capsulatum and other fungal pathogens share a number of cell wall and cytoplasmic antigenic determinants (1, 6, 8, 9, 21, 31). H. capsulatum may also contain unique components that are not shared with other organisms. Monoclonal antibody (MAB) technology has proved useful in identifying single antigenic determinants (epitopes), even in complex mixtures. This technology has also been used recently for the production of MAbs against histoplasmin (33), Candida albicans (26, 29, 36, 42), Blastomyces dermatitidis (50), and Cryptococcus neoformans (7, 10).

MAbs might be useful for identification of specific Histoplasma antigens which could be used to improve the specificity of the serologic tests, as well as for identification of the cell-mediated immune responses in histoplasmosis. Herein we report our findings on the preparation and characterization of four MAbs against H. capsulatum yeast cell antigens.

MATERIALS AND METHODS

Antigens. A whole yeast cell extract of H. capsulatum G217B was prepared as previously described (44). In brief, yeast cells were grown in brain heart infusion broth (Difco Laboratories, Detroit, Mich.) supplemented with 1% glucose. Flasks were incubated at 37°C on a Gyrotory shaker (New Brunswick Scientific Co., Inc., New Brunswick, N.J.) with a shaking speed of 150 rpm. The yeast cell cultures were centrifuged and washed twice in 0.02 M Tris hydrochloride buffer (pH 7.5) at 4°C. The packed cells were suspended in equal volumes of 0.1 M Tris hydrochloride buffer (pH 7.8) and transferred to a Braun homogenizer glass bottle (B. Braun Co., Helsungten, Federal Republic of Germany) containing an equal amount of acid-washed glass beads (diameter 0.45 to 0.55 mm). The cells were broken in the Braun homogenizer by shaking for two 1-min periods. Unbroken cells and debris were removed by centrifuging the suspension at 20,000 × g for 20 min. The supernatant was collected, and the pellet was resuspended in the same buffer and centrifuged at 30,000 × g for 30 min. The two supernatants were pooled to make the H. capsulatum whole yeast cell extract.

The yeast form of clinical isolates of B. dermatitidis, C. albicans, C. neoformans, Sporothrix schenckii, and H. capsulatum G217B, A811 (from the Centers for Disease Control, Atlanta, Ga.), and P-IN (isolate from Indianapolis, Ind.) were grown in brain heart infusion broth supplemented with 1% glucose. Flasks were incubated in the Gyrotory shaker for 48 h at 37°C and 150 rpm. Cells were harvested by centrifugation at 8,000 × g for 15 min and washed twice in normal saline. The cells were killed by incubation with 3.7% Formalin at 25°C for 24 h and washed in normal saline, and the nonviability was verified by culture at 37°C on brain heart infusion agar for 72 h. A 10% suspension of the killed cells was suspended with an equal volume of glass beads in normal saline and sonicated in an ultrasonicator (Model U-20; Blackstone Corp., Jamestown, N.Y.) for 4 to 6 h. Soluble whole yeast cell extracts were prepared by centri-
fusing the sonicated cell suspension at 8,000 × g for 15 min, and the supernatants were collected.

The mycelial form of clinical isolates of Aspergillus fumigatus, Coccidioides immitis, Paracoccidioides brasiliensis, and H. capsulatum G217B, A811, and P-IN were grown on Sabouraud or tryptic soy agar plates at 25°C for 48 h (A. fumigatus), 4 weeks (C. immitis and P. brasiliensis), or 6 weeks (H. capsulatum). Spores of each culture were harvested in normal saline, kept in 3.7% Formalin solution for 25 h, and then washed 6 to 10 times in normal saline. A 10% spor suspension from the nontuable cultures was prepared in normal saline and was sonicated with equal amounts of glass beads in a batch sonicator for 4 to 6 h. Disrupted spore cell suspension were centrifuged at 8,000 × g for 15 min, and supernatant fluids were collected.

Commercially available antigens from H. capsulatum, B. dermatitidis, C. immitis, and A. fumigatus which were prepared for use in immunodiffusion assays were obtained from American Micro Scan, Sacramento, Calif. Tuberculin was obtained from the Mycobacteriology Laboratories, National Jewish Hospital, Denver, Colo. Preparation of MAbs. Female BALB/c mice (Harlan Sprague Dawley, Madison, Wis.) 8 to 10 weeks old, were immunized with H. capsulatum whole yeast cell extracts as follows. Mice were injected intraperitoneally on day 1 with 0.5 ml of the extract (100 µg of protein per mouse) emulsified in Freund incomplete adjuvant (Difco) and on day 14 with 0.5 ml of the extract in sterile saline. On day 21, the mice were bled and the sera were tested by enzyme-linked immunosorbent assay (ELISA) or radioimmunoassay for antibody against a soluble yeast cell extract of H. capsulatum G217B. If the sera were positive, the mice were boosted intraperitoneally on day 28 with 0.5 ml of the extract (100 µg of protein per mouse) in normal saline, and on day 32 the mouse spleen cells were used for fusion.

Spleen cells from mice with elevated levels of antibodies to H. capsulatum were fused with Sp2/0 myeloma cells in the log phase in a ratio of 10:1, using 45% polyethylene glycol (J. T. Baker Chemical Co., Phillipsburg, N.J.) as described by Zola and Brooks (51). The fused cells were plated into 96-well plates and incubated at 37°C in 7% CO2. Initial screening for antibody activity against H. capsulatum whole yeast cell extract was done 14 to 16 days after fusion by using a solid-phase radioimmunoassay. In this assay, rabbit anti-mouse immunoglobulin G (IgG) plus IgM (Accurate Chemical and Scientific Corp., Westbury, N.Y.) were used, followed by 125I-labeled protein A. Positive hybridomas were cloned twice by the limiting dilution method. MAbs were produced in large quantities in 75-cm2 tissue culture flasks and in mouse ascitic fluid. The MAbs were then purified by ammonium sulfate precipitation and/or affinity chromatography, using carbodiimide (CDI)-activated agarose CL-6B (Pierce Chemical Co., Rockford, Ill.) charged with rabbit anti-mouse immunoglobulins (Accurate Chemical and Scientific). The MAbs were eluted with 100 M MPO-4 NaHPO4 buffer (pH 2.7).

Solid-phase radioimmunoassay. Polystyrene, flat-bottom 96-well plates were coated with 0.1 ml of the desired antigen in 0.01 M Tris hydrochloride (pH 7.0). Unbound sites were blocked with 0.1 ml of 5% bovine serum albumin (BSA) in 0.01 M Tris hydrochloride (pH 7.0). Next, 0.1 ml of the test antibody diluted in 5% BSA in 0.1 M Tris-saline (pH 8.0) was added to the wells. Wells receiving antibodies reactive with the solid-phase antigen were identified by adding 0.1 ml of rabbit anti-mouse IgG or IgM diluted 1:1,000 in 5% BSA in 0.1 M Tris-saline (pH 8.0). Finally, each well received 0.1 µg of 125I-labeled protein A in 5% BSA in 0.1 M Tris-saline (pH 8.0) (50,000 cpm per well). For each test, the plates were incubated at 37°C for 1 h, the supernatants were aspirated, and the wells were washed three times with phosphate-buffered saline-Tween 20. Radioactivity (counts per minute [cpm]) was determined with a Beckman gamma 4000 counter. All specimens were tested in triplicate.

ELISA. A H. capsulatum whole yeast cell extract (0.1 ml containing 100 µg of protein) in 0.1 M Na2CO3 buffer (pH 9.6) was added to each well of a 96-well plate. Unbound sites in each well were blocked with 0.1 ml of 5% BSA in 0.01 M Tris hydrochloride buffer (pH 7.0). Next, the test antibody suspension (0.1 ml) in 5% BSA in 0.1 M Tris-saline buffer (pH 8.0) was dispensed into each well. A 1/40 dilution of alkaline phosphatase-conjugated anti-mouse immunoglobulin (Accurate Chemical and Scientific) in 0.1 M Tris-saline buffer in 5% BSA (pH 8.0) was dispensed into each well. Finally, a 0.1-ml portion of p-nitrophenyl phosphate (Sigma Chemical Co., St. Louis, Mo.; 1 mg/ml in Na2CO3 buffer, pH 9.2) was dispensed into each well. For each step, plates were incubated at 37°C for 1 h, aspirated, and washed three times with phosphate-buffered saline-Tween 20. Reactivity was measured spectrophotometrically with a Multiscan MC MR 600 automatic ELISA reader (Dynatech Instruments, Inc., Torrance, Calif.). All samples were tested in triplicate.

Western protein blotting (immunoblotting). Immunoblotting was performed as described by Batteiger et al. (3), based on modification of earlier procedures of Laemmli (24). In brief, separation gels (9 by 14 by 0.15 cm) containing 10% acrylamide, 0.375 M Tris hydrochloride (pH 8.8), and 0.1% sodium dodecyl sulfate were prepared in a Bio-Rad slab gel apparatus (Model 220). Proteins (antigens) were solubilized in 5% (wt/vol) sodium dodecyl sulfate–1% (vol/vol) 2-mercaptoethanol–1.25% (vol/vol) glycerol in 2.5 mM Tris hydrochloride (pH 6.8). The solubilization mixtures were incubated at 100°C for 10 min and cooled to 25°C. Antigenic extracts were standardized to contain 20 µg of protein per ml per lane. Electrophoresis conditions were those described by Batteiger et al. (3). Each strip was incubated with 125I-labeled protein A (95,000 cpm per strip).

Subtyping of the MAbs. The subtyping of the MAbs against H. capsulatum was performed as specified in the instructions of a commercially available kit (ICN Immunologicals, Lisle, Ill.). In brief, rabbit anti-mouse IgG1, IgG2a, IgG2b, IgG3, and IgM were placed in the peripheral wells of a seven-well immunodiffusion plate. The MAb to be tested was placed in the central well. The plates were incubated at 25°C for 48 h. All tests were performed in triplicate, and all MAbs were tested at least twice.

RESULTS

Immunoglobulin class and subclass of MAbs. Two fusions between the Sp2/0 myeloma cell line and spleen cells from female BALB/c mice immunized with soluble whole yeast cell extract of H. capsulatum G217B resulted in the production of four MAbs against H. capsulatum which were obtained from four hybrid cell lines. The immunoglobulin class and subclass of these four MAbs were determined by immunodiffusion, using a commercially available kit, as described above. They belonged to three subclasses: MAb 1 was of the subclass IgG2b, MAbs 2 and 4 were of the subclass IgG3, and MAb 3 was of the subclass IgG1.

Specificity of MAbs. Antigenic preparations (yeast and/or mycelial form) of A. fumigatus, B. dermatitidis, C. albicans,
C. immitis, C. neoformans, H. capsulatum (strains G217B, A811, and P-IN), P. brasiliensis, S. schenckii, and Mycobacterium tuberculosis were prepared as described above. The reactivity of different dilutions of these antigenic preparations was verified by solid-phase radioimmunoassay with homologous serum from rabbits immunized with fungal and mycobacterial pathogens. Optimal dilutions were selected to test the specificity of the four MAbs in solid-phase radioimmunoassay. Pathogens used were divided into three groups. Group 1 consisted of closely related fungal pathogens: H. capsulatum, B. dermatitidis, C. immitis, and P. brasiliensis. Group 2 consisted of other fungal pathogens: A. fumigatus, C. albicans, C. neoformans, and S. schenckii. Group 3 consisted of M. tuberculosis. To simplify and standardize the reactivity of the four MAbs, a reactivity index was developed. The reactivity index was calculated by dividing the reactivity (cpm) of the MAb with an antigen by the reactivity (cpm) of the same MAb with the no-antigen control. A reactivity index greater than twice that of the no-antigen control was considered positive. Antibody activity of MAbs against H. capsulatum was not determined by titration but rather by testing a single concentration. Also, standardization to allow accurate quantitation of antibody content was not performed. Thus, the immunoglobulin concentrations of different MAb preparations or activity of a single MAb against different antigens could not be precisely determined. Nevertheless, in our experience, measurements of antibody activity based on results of testing a single dilution correlate well with endpoint antibody titers and probably provide a semiquantitative assessment of antibody activity. The reactivity indices of MAbs 1 and 2, which showed positive indices only with the yeast cell antigens of H. capsulatum (reactivity indices, 92 and 17, respectively) and B. dermatitidis (reactivity indices, 16 and 22, respectively), are shown in Table 1. Both MAbs showed negative reactivity indices (0.6 to 2) with the other pathogens. The reactivity indices of MAbs 3 and 4 are shown in Table 2. MAb 3 showed positive reactivity indices only with the yeast forms of H. capsulatum, B. dermatitidis, C. albicans, P. brasiliensis, and S. schenckii, whereas MAb 4 had positive reactivity indices with the yeast antigens of H. capsulatum (index, 52) and B. dermatitidis (index, 8), the mycelial antigen of C. immitis (index, 4), the yeast and mycelial antigens of C. albicans (indices, 12 and 6, respectively), the mycelial antigen of A. fumigatus (index, 4), and the tuberculin antigen of M. tuberculosis (index, 3). MAbs 1 and 2 were more specific to H. capsulatum antigens than the broadly cross-reacting MAbs 3 and 4.

### Reactivity of MAbs Against Different Strains of H. capsulatum

For partial evaluation of the strain or serotype specificity of the MAbs prepared against H. capsulatum, their reactivity with antigens from three different H. capsulatum strains was tested in solid-phase radioimmunoassay. All four MAbs showed positive reactivity indices (8 to 66) with the mycelial and yeast antigens of H. capsulatum G217B (Table 3). MAbs 1, 2, and 3 had positive reactivity indices (3, 3, and b, respectively) with the mycelial antigen of H. capsulatum A811. All MAbs showed positive reactivity indices (6 to 15) with the yeast antigen of H. capsulatum P-IN, but only MAb 2 reacted with the mycelial antigen.

### Characterization of MAbs by Western Immunoblot Assay

The MAbs were further characterized in a Western immunoblot assay. The electrophoresed yeast cell extract of H. capsulatum was reacted with the MAbs. The reactivity patterns of MAbs 1 and 2 and that of the serum from a rabbit immunized with H. capsulatum (positive control) are shown in Fig. 1. MAb 1 showed the most intense reactivity; it yielded five bands with apparent molecular masses of 72, 62,
TABLE 3. Reactivity indices of MAbs with mycelial and yeast cell extracts of different strains of \textit{H. capsulatum}

<table>
<thead>
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<th>MAb</th>
<th>\text{Reactivity index}\textsuperscript{a} with \textit{H. capsulatum} strain:</th>
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<tr>
<td></td>
<td>\text{G217B}</td>
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<td>None\textsuperscript{b}</td>
<td>1.7</td>
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<tr>
<td>NRS</td>
<td>96.6</td>
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<tr>
<td>IRS</td>
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<td>1</td>
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<td>2</td>
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<td>3</td>
<td>23.4</td>
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\textsuperscript{a} Reactivity (cpm) of MAb with antigen/reactivity (cpm) of the same MAb in control (no antigen). \text{M}, Mycelial extract; \text{Y}, yeast cell extract.

\textsuperscript{b} NRS, Normal rabbit serum control; IRS, homologous positive immune rabbit serum control.

21, 16, and 13 kilodaltons (kDa), which were more visible in the original autoradiograph than in the figure, and two broad bands with apparent molecular masses of 44 and 28 kDa. MAb 2 yielded three distinct bands with apparent molecular masses of 72, 62, and 21 kDa and two broad bands with apparent molecular masses of 44 and 28 kDa. Serum from the rabbit immunized with \textit{H. capsulatum} also showed a highly intense reaction with the electrophoresed \textit{H. capsulatum} antigens. MAb 3 yielded one distinct band and two broad bands with apparent molecular masses of 72, 44, and 28 kDa, whereas MAb 4 did not react in the Western immunoblot assay.

**DISCUSSION**

Definitive diagnosis of histoplasmosis is made only by the isolation of \textit{H. capsulatum} from clinical specimens. However, culture methods are not positive for all patients with histoplasmosis. Wheat et al. (45, 48) reported that cultures were positive for only 65 to 90\% of patients with cavitary or disseminated histoplasmosis and were generally negative for the more common self-limited syndromes. Also, there is at least a 2- to 6-week delay before \textit{H. capsulatum} can be identified by culture, leading to serious consequences for some patients with severe infection. Moreover, invasive procedures are often required to obtain biopsies from patients with negative cultures from sputum or blood. More recently, the availability of a rapid radioimmunoassay for detection of \textit{H. capsulatum} antigen in urine and blood has permitted prompt and specific diagnosis of severe cases of histoplasmosis (49), but this test has not been very useful in milder cases. Serologic tests have proved to be more sensitive; more than 90\% of patients with histoplasmosis, including those with cavitary or disseminated, as well as self-limited, disease, were positive in complement fixation tests (39, 46-48). Histoplasma serological tests are comparatively rapid, since results can be obtained in 8 to 24 h. By using sensitive radioimmunoassay, \textit{Histoplasma} antibodies can be detected as early as 3 weeks after the onset of infection (48). However, the increased sensitivity of \textit{Histoplasma} serological tests is accompanied by a decrease in specificity. Such false-positive results are attributed to a number of factors, one of which is the use of nonstandardized antigen preparations which contain antigens shared by other fungal and bacterial pathogens. The presence of these antigens has been recognized and reported by many investigators (5, 11-13, 15, 16, 18-20, 23, 27, 28, 30, 32, 37, 46). Attempts to purify antigenic components that are specific to \textit{H. capsulatum}, using biochemical or chromatographic techniques, have had limited success (1, 2, 4, 28, 35, 37).

MAbs could be used in affinity purification of specific antigens to improve the accuracy of serologic tests. Reiss et al. (33) produced MAbs against \textit{H. capsulatum} by using a mycelial-phase antigen of the organism. The MAbs described in that report appear to differ from those discussed in this study. Reiss et al. used a purified M antigen from mycelial-phase organisms for vaccination, whereas we used the yeast-phase antigen. One of the MAbs (CB4) of Reiss et al. was an IgM antibody which recognized a polysaccharide antigen, and the other was an IgG antibody which recognized a protein dimer. Our four MAbs were all IgG antibodies, but we did not characterize the antigens recognized by our MAbs. MAb 1 and 2 formed smears at the top of the gel when tested by Western immunoblot, as did MAb CB4 of Reiss et al. Reiss et al. attributed the smearing to the polysaccharide nature of the antigen recognized by MAb CB4. We have demonstrated that this smear was caused by a glycoprotein in other studies using Western immunoblot techniques with periodate- and protease-treated whole yeast extracts and polyclonal rabbit antibodies to \textit{H. capsulatum} (unpublished observations). The IgM MAb CB4 of Reiss et al. formed a precipitin line with the \textit{H. capsulatum} M antigen, whereas the IgG MAb EC2 was not reactive by immunodiffusion. Our MAbs were not reactive with \textit{H. capsulatum} H or M antigens by immunodiffusion (data not shown). MAb CB4 of Reiss et al. cross-reacted with \textit{C. immitis} and \textit{B. dermatitidis}, but EC2 was nonreactive by immunodiffusion. Our MAbs 1 and 2 cross-reacted by radioimmunoassay with \textit{B. dermatitidis}, while MAbs 3 and 4 were more broadly cross-reactive (MAb 3 with \textit{B. dermatitidis}, \textit{P. brasiliensis}, \textit{C. albicans}, and \textit{S. schenckii} and MAb 4 with \textit{C. immitis}, \textit{C. albicans}, \textit{A. fumigatus}, and \textit{M. tuberculosis}). Finally, MAbs CB4 and EC2 of Reiss et al. were only weakly reactive by ELISA, using microdilution wells coated with the purified M antigen, whereas our MAbs were highly reactive by ELISA and radioimmunoassay with histoplasmin and soluble yeast-phase antigens of \textit{H. capsulatum}.

MAbs 1 and 2 prepared in this study recognized at least...
one epitope of *H. capsulatum* that is shared with the yeast antigen of *B. dermatitidis*. This is the first report of the production of MAbs against the yeast antigen of *H. capsulatum*. Previous studies with polyclonal sera also found common antigens between *H. capsulatum* and *B. dermatitidis* (12, 32, 37). MAbs 3 and 4 showed cross-reactivity with several other fungal pathogens. MAbs 3 cross-reacted with the yeast antigens of *B. dermatitidis*, *P. brasiliensis*, *S. schenckii*, and *C. albicans*. MAb 4 cross-reacted with the yeast antigen of *B. dermatitidis*, the mycelial antigens of *C. immitis* and *A. fumigatus*, and the yeast and mycelial antigens of *C. albicans* and *M. tuberculosis*. These results suggest that the two epitopes recognized by MAbs 3 and 4 are different from each other and from those recognized by MAbs 1 and 2. Broad cross-reactivity between antigens of *H. capsulatum* and other fungal and mycobacterial pathogens have been reported in several other studies (11–13, 19, 20, 30, 32, 46). However, none of these studies determined whether cross-reactivity was due to one or more antigens, because polyclonal antisera were used and not MAbs.

Recognition of different *H. capsulatum* serotypes was revealed previously (19). Data from that study indicated the presence of at least five serotypes of *H. capsulatum*. Our four MAbs exhibited possible serotype or strain specificity. All four antibodies recognized the yeast antigens of the homologous strain (G217B) and an isolate from a patient, while none reacted with the yeast antigen of strain A811. MAbs 1, 2, and 3 reacted with the mycelial antigen of strain A811; however, since evaluation of serotype specificity was not an objective of this study, thorough analysis of a large number of strains was not undertaken. These results suggest that the four MAbs recognize serotype- or strain-specific epitopes of *H. capsulatum*.

The four MAbs reacted differently in Western immunoblot assay with electrophoresed *H. capsulatum* antigens. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis of the ultrasonic whole yeast cell extract of *H. capsulatum* revealed the presence of a composite mixture of multiple components with different molecular masses. A previous study (23) also demonstrated the presence of multiple components in several preparations of *H. capsulatum* by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. The present study and other studies (23, 33) demonstrated certain limitations of Western blotting with *H. capsulatum* antigens. Diffuse smudging (23, 33) may obscure discrete bands within the smudge. This smear is thought to be caused by the electrophoretic properties of polysaccharide complexes or polymers (33). Our more recent unpublished experience with Western blotting with polyclonal antibodies and periodate and protease-treated antigens indicates that the smear disappears with periodate treatment, supporting the importance of the polysaccharide as a cause for smearing. Also, certain important polysaccharide antigens may not electrophorese or may not bind to nitrocellulose membranes. Despite these limitations, a few conclusions can be drawn. Different patterns of reactivity were shown by MAbs 1 and 2, indicating that they recognized two different epitopes. MAb 3 reacted in the Western immunoblot, whereas MAB 4 did not, confirming an earlier conclusion that these two MAbs recognize different epitopes. The presence of multiple bands in the Western immunoblot assay with MAbs might be explained in three different ways. First, these antibodies might not be truly monoclonal. Second, the epitopes recognized by these antibodies might be expressed in more than one of the antigenic components of *H. capsulatum*. Third, the antigens containing these epitopes might be polymers, which were broken down into fragments of different molecular masses. The purity of the MAbs was evident in more than one experiment. First, each MAb was cloned at least twice before production on a large scale. Also, each MAb showed only one precipitin band with the subclass anti-immunoglobulin. It is unlikely that polyclonal antibodies would all belong to the same immunoglobulin subclass. Recognizing that the whole yeast cell extract of *H. capsulatum* is a mixture of components of different molecular masses, the possibility is great that an epitope recognized by an MAb might be present on different antigenic components or subunits. Others have also demonstrated multiple bands with MAbs in the Western immunoblot assay (36).

In conclusion, four MAbs against the yeast antigen of *H. capsulatum* were prepared and characterized. Unique features of these antibodies indicate that they recognize different epitopes and may demonstrate some degree of serotype specificity. MAbs 1 and 2 showed more specificity to *H. capsulatum*, as they cross-reacted only with the yeast antigen of *B. dermatitidis*. MAbs 3 and 4 showed broader cross-reactivity. MAbs may prove useful in the development of more specific immunodiagnostic tests for histoplasmosis.

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LITERATURE CITED


