JOURNAL OF CLINICAL MICROBIOLOGY, May 1997, p. 1071–1076 Vol. 35, No. 5
0095-1137/97/$04.00+0

Molecular Cloning and Characterization of a Recombinant *Histoplasma capsulatum* Antigen for Antibody-Based Diagnosis of Human Histoplasmosis

R. CHANDRASHEKAR,[1,2]* K. C. CURTIS,[1] B. W. RAWOT,[1]†
G. S. KOBAYASHI,[1,2] AND G. J. WEIL[1,2]

Departments of Medicine[1] and Molecular Microbiology,[2] Barnes-Jewish Hospital and Washington University School of Medicine, St. Louis, Missouri 63110

Received 9 September 1996/Returned for modification 28 October 1996/Accepted 3 February 1997

Immunological cross-reactivity among fungi has hampered the development of specific serodiagnostic assays for histoplasmosis. We report the molecular cloning and characterization of a *Histoplasma capsulatum* cDNA (GH17) that encodes an antigen with immunodiagnostic potential. GH17 is an 810-bp cDNA which encodes a protein of 211 amino acid residues. The GH17 sequence has almost no significant homology with other sequences in GenBank. Southern blot analysis suggests that GH17 is confined to a single location in the genomic DNA of *H. capsulatum*. Immunoblots indicated that the protein product of GH17 (expressed as a 140-kDa β-galactosidase fusion protein) was recognized by antibodies in 18 of 18 sera from histoplasmosis patients, but not by antibodies in sera from patients or animals infected with other fungi. GH17 was expressed in a prokaryotic expression vector, pPROEX-1, and recombinant protein was purified by preparative electrophoresis. Antibodies raised to this protein bound to a 60-kDa native antigen in immunoblots of *H. capsulatum* yeast antigen extract. These results suggest that GH17 encodes an *H. capsulatum* antigen that may be useful for the diagnosis of histoplasmosis in humans.

Histoplasma capsulatum is a pathogenic dimorphic fungus that grows as multicellular mycelia in nature and as unicellular budding yeasts in humans and animals (1). The inhalation of airborne propagules results in the morphological transformation to the yeast form, which causes pulmonary infection and occasional progressive disease, particularly in immunosuppressed patients (10). Histoplasmosis is highly prevalent in the Ohio and Mississippi valleys in the United States (1, 8, 34), and it is also widely distributed in Latin America (18), southern Europe (34), Asia (8), Australia (29), and Africa (18).

The diagnosis of histoplasmosis in humans is often suggested by the results of a careful clinical evaluation and radiologic studies, but laboratory tests are necessary for confirmation (16). Isolation of the organism from blood or tissue provides a definitive diagnosis. Serological tests are also important tools for diagnosing histoplasmosis. The most widely available tests are the immunodiffusion assay, which detects antibodies to heat-sensitive glycoproteins called H and M antigens, and the more sensitive complement fixation test, which traditionally is performed with yeast and mycelial antigens (2–4, 11). More sensitive antibody assays, such as radioimmunoassay and enzyme immunoassay, have been used to detect immunoglobulin M (IgM) and IgG antibodies to crude fungal extracts (9, 23, 31, 38).

Attempts to develop antibody serology tests for diagnosis of histoplasmosis have been hampered by poor specificity caused by immunologic cross-reactivity between various fungal species. Much of this cross-reactivity is due to nonprotein determinants (e.g., carbohydrates and phosphorylcholine) which are present in many components of crude antigen extracts prepared from the yeast and mycelial stages of *H. capsulatum*. Thus, the detection of antibodies to defined native or recombinant antigens may improve the specificity and sensitivity of antibody assays. The advent of recombinant-DNA technology allows the efficient screening of DNA expression libraries for the identification of potentially useful recombinant antigens and the production of antigens in large quantities for the development of species-specific antibody assays. Although this approach has recently been used successfully to develop improved antibody assays for nematode infections (5, 6, 24, 25), to date, it has not been widely applied to fungal serology. Therefore, the purpose of the present study was to identify and characterize recombinant *H. capsulatum* antigens that might be useful for the immunodiagnosis of histoplasmosis.

MATERIALS AND METHODS

Fungi and culture conditions. *H. capsulatum* G217B, a North American isolate, was obtained from the American Type Culture Collection (ATCC 26032, Rockville, Md.). Mycelial-phase organisms were cultured in a shaking water bath at 25°C in broth containing 2% glucose and 1% yeast extract. Yeast-phase organisms were grown at 37°C in HMM broth (HMM consists of F-12 nutrient mixture with L-glutamine and phenol red without sodium bicarbonate) (Gibco-BRL, Gaithersburg, Md.) supplemented with 18.2 g of glucose and 1.0 g of glutamic acid per liter and adjusted to pH 7.5 (17).

Human and animal sera. Human sera were from patients with well-documented histoplasmosis ($n = 18$), coccidioidomycosis ($n = 12$), and candidiasis ($n = 5$). Coccidioidomycosis sera were generously provided by Demosthenes Pappagianis, University of California School of Medicine (Davis). The histoplasmosis sera were obtained from patients with acute and chronic disease and from patients with disseminated infections (indicated by positive bone marrow and/or blood cultures) associated with AIDS. The laboratory diagnosis of histoplasmosis infection was based on culture and biopsy results and/or serology tests (immunodiffusion and complement fixation with yeast and mycelial antigens). Blastomycosis sera from humans ($n = 5$) and dogs ($n = 6$) with documented clinical infections and from rabbits immunized with *Blastomyces dermatitidis* antigens or whole yeast cells ($n = 3$) were a gift from Gene Scalerone (Idaho State College, Pocatello).

Control human sera were obtained from healthy residents of St. Louis, Mo. A histoplasmosis serum pool was prepared with sera ($n = 12$) from patients with proven histoplasmosis.

Isolation of *H. capsulatum* DNA. Genomic DNA from yeast cells was isolated essentially as previously described (17, 35). Briefly, yeast cells were pelleted and

* Corresponding author. Present address: Heska Corporation, 1825 Sharp Point Dr., Fort Collins, CO 80525. Phone: (970) 493-7272. Fax: (970) 493-7333. E-mail: Chandrr@Heska.com.
† Present address: Suite 304, 400 N. Allen Dr., Allen, TX 75013.

resuspended in TE buffer (10 mM Tris [pH 8.0], 1 mM EDTA). Sodium dodecyl sulfate (SDS) was added to a 1% final concentration. DNA was extracted with phenol-chloroform, ethanol precipitated, and washed with 70% ethanol.

Mouse sera. Antibodies to yeast antigen or to a histidine fusion protein of the recombinant clone GH17 (GH17-his; see below) were produced in 6-week-old female BALB/c mice by footpad injection of 10 μg of yeast antigen or purified GH17-his in Freund's complete adjuvant, followed by a second injection of antigen in Freund's incomplete adjuvant 4 weeks later. Sera were collected 1 week after the booster immunization.

Yeast antigen. Yeast cells were suspended in 0.01 M Tris buffer (pH 8.3) containing protease inhibitors (1 mM phenylmethylsulfonyl fluoride, 1 mM EDTA, 25 μg of *N*-tosyl-L-phenylalanine chloromethyl ketone per ml, and 25 μg of *N-a-p*-tosyl-L-lysine chloromethyl ketone per ml) (all from Sigma Chemical Co., St. Louis, Mo.). The yeast homogenate was rocked at 4°C overnight and centrifuged at 15,000 × *g* for 10 min. The protein concentration in the supernatant was measured with a commercial bicinchoninic acid kit (Pierce Chemical Co., Rockford, Ill.).

Screening of a gene expression library and selection of recombinant clones. A λgt11 cDNA library was custom synthesized (Clontech Laboratories Inc., Palo Alto, Calif.) with poly(A)$^+$ mRNA derived from the mycelial stage of *H. capsulatum* G217B. This library has a recombinant frequency of over 90% after amplification. The DNA insert size range is 0.6 to 4.5 kb, with an average of 1.6 kb. The library was immunoscreened to identify *H. capsulatum*-specific clones essentially as previously described (5, 6). Clones that were reactive with antibodies in the histoplasmosis serum pool but not with a normal human serum pool were selected and purified by repeated cycles of immunoselection. The reactivity of serum pools to fusion proteins expressed by purified recombinant phage was studied by plaque dot immunoblot analysis as previously described (5). PCR was employed to amplify the cDNA inserts of selected recombinant λgt11 clones with the GenAmp DNA amplification kit (Perkin-Elmer Cetus, Norwalk, Conn.) as previously described (32). DNA dot hybridization was performed with peroxidase-labeled DNA fragments (14) to assess homology between the selected clones.

Southern blot analysis and DNA sequencing. *H. capsulatum* genomic DNA (5 μg) was cut with selected restriction endonucleases. Digestion products were electrophoresed in a 1% agarose gel and transferred to a Hybond-N+ nylon transfer membrane (Amersham, Arlington Heights, Ill.) by standard techniques, and blots were probed with the labeled cDNA insert of GH17 (27).

The λgt11 DNA purified from GH17 was digested with *Eco*RI and ligated into pBluescript II SK− (Stratagene Cloning Systems, La Jolla, Calif.) by standard methods (27), and plasmid DNA was prepared for sequencing. The dideoxynucleotide chain termination method (33) was used for double-stranded DNA sequencing, with the TaqTrack sequencing system (Promega Corporation, Madison, Wis.) with T3 and T7 pBluescript primers and synthetic oligonucleotides.

The PC/GENE DNA sequence analysis software (Intelligenetics, Mountain View, Calif.) and the BLAST program (National Center for Biotechnology Information, National Library of Medicine, National Institutes of Health, Bethesda, Md.) were used to analyze nucleotide and deduced amino acid sequences and to assess their homology with sequences in the GenBank database.

Expression and purification of GH17. The cDNA insert of the recombinant clone GH17 was subcloned directionally into the plasmid expression vector pPROEX-1 (Gibco-BRL) to produce a fusion protein containing six histidines. The fusion protein (GH17-his) was purified from bacterial lysates by continuous-elution electrophoresis with a Prep Cell (Bio-Rad Laboratories, Hercules, Calif.). Briefly, a 10-ml overnight culture of *Escherichia coli* (BL21 strain) cells containing the recombinant plasmid GH17 was inoculated into 700 ml of NZCYM medium (Gibco-BRL) containing 50 μg of ampicillin per ml (Sigma). Cultures were grown at 37°C with shaking to optical density at 600 nm of 1.0. Isopropyl-β-D-thiogalactopyranoside (IPTG) (final concentration, 0.3 mM) was then added, and the culture was grown for an additional 5 h, after which the cells were pelleted and resuspended in a 1:50 (vol/vol) solution of lysis buffer (10 mM Na_2HPO_4, 30 mM NaCl, 0.25% Tween 20, 10 mM EDTA, 10 mM EGTA [pH 7.0]). The cells were frozen at −20°C overnight, thawed in cold water, and lysed by mild sonication. Cellular debris was removed by centrifugation at 10,000 × *g* for 15 min. A 12% polyacrylamide gel was poured per the manufacturer's protocol for the Bio-Rad model 491 Prep Cell. Ten milliliters of sample in 1:1 loading buffer (0.0625 M Tris-HCl [pH 6.8], 10% glycerol, 0.025% bromphenol blue) was loaded, and the gel was run for 8 h at 12 W of constant power. Three-milliliter fractions were collected and run on SDS-polyacrylamide gel electrophoresis (SDS-PAGE) (12% polyacrylamide) minigels (22). Western blots (36) were performed with the histoplasmosis human serum pool to identify fractions that contained the GH17 fusion protein. Three consecutive fractions containing the band of interest were selected, pooled, and dialyzed against phosphate-buffered saline (PBS), pH 7.2. The dialyzed protein was concentrated with a membrane concentrator (Centriplus; Amicon, Beverly, Mass.), and the protein concentration was measured with a commercial bicinchoninic acid kit (Pierce Chemical Co.)

Immunoblot analysis of recombinant fusion proteins. *E. coli* Y1090 was infected at high density with recombinant phage on a thin layer of agarose over Luria-Bertani agar to achieve confluent lysis, and synthesis of the fusion proteins encoded by the cDNA inserts was induced with IPTG-impregnated filters. The agarose layer containing bacterial lysate and fusion protein was then gently scraped off and dissolved in SDS-PAGE sample buffer. SDS-PAGE was performed as described by Laemmli (22) at 135 V in 8% reducing gels. After SDS-PAGE, the proteins were transferred electrophoretically (36) to nitrocellulose membranes. The membranes were then incubated with monoclonal antibody to β-galactosidase (Promega Biotec, Madison, Wis.) or with canine or human sera diluted 1:500 in containing 0.05% Tween 20 (Sigma) (PBS-T) for 3 h at 37°C. Membranes were washed in PBS-T and incubated with alkaline phosphatase conjugated goat anti-mouse, anti-dog or anti-human IgG (Promega) for 1 h at 37°C. After they were washed, the membranes were developed with Nitro Blue Tetrazolium–5-bromo-4-chloro-3-indolylphosphate.

Immunoblot analysis was carried out with *H. capsulatum* yeast extract to identify the native antigen(s) that corresponds to GH17. Yeast extract was separated by SDS-PAGE on 5 to 25% gradient slab gels and processed as described above with mouse antibody to GH17-his.

Nucleotide sequence accession number. The sequence of GH17 has been deposited in the GenBank database under accession number U27588.

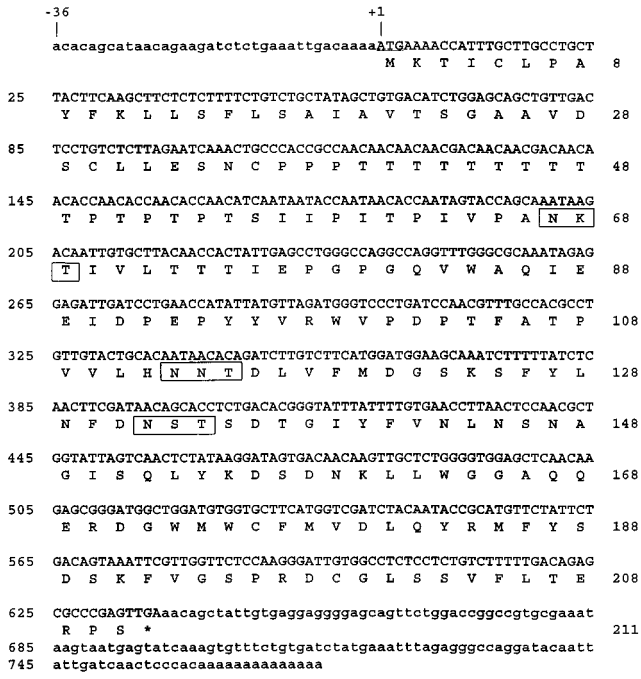

FIG. 1. The sequence of the cDNA insert of GH17 is shown (5′ to 3′), with the predicted amino acid sequence encoded by its ORF. Nucleotides are numbered (left margin), and nucleotides 5′ to the initiation codon (underlined) are indicated by negative numbers. Deduced amino acid residues are numbered (right margin). The coding region begins with a methionine codon 36 bp from the 5′ end and ends at base 633 with a stop codon (the asterisk indicates the end of the encoded product). The putative N-glycosylation sites are boxed.

RESULTS

Selection of λgt11 clones that express *H. capsulatum*-specific antigens. Approximately 500,000 phage plaques from an *H. capsulatum* mycelial-phase cDNA expression library were immunoscreened with a histoplasmosis serum pool and a control serum pool made from sera obtained from healthy residents of St. Louis who had no history of histoplasmosis. Twenty clones selected in the initial screen were rescreened with individual histoplasmosis sera. Eight highly immunoreactive clones were identified, and these were again tested by plaque dot immunoblots with sera from patients with other fungal infections. Four clones were selected for further study. DNA dot hybridization studies showed that all four clones hybridized to each other, even under high-stringency conditions (data not shown). The four clones, designated GH2, GH17, GH22, and GH23, produced a similar-size β-galactosidase fusion protein with an

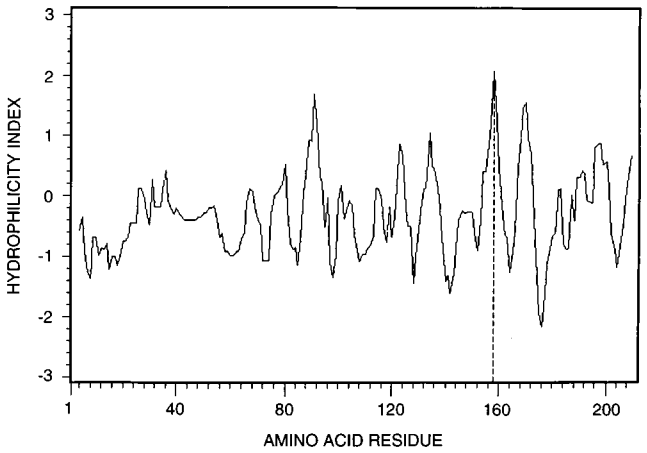

FIG. 2. Hydropathy plot of the protein encoded by GH17. Hydropathy analysis was performed by the method of Hopp and Woods (13a). Hydropathy values were averaged for a window of six amino acid residues. Positive numbers indicate hydrophilicity. The point of highest hydrophilicity (average hydrophilicity [2.08] is between residues 155 and 160) is marked with a broken vertical line.

apparent M_r of 140,000, in contrast to 116 kDa for unfused β-galactosidase (data not shown).

Molecular characterization of recombinant *Histoplasma* clones. The cDNA inserts of clones GH2, -17, and -22 were sequenced. All three clones contained identical 5′ ends and an identical 633-bp open reading frame (ORF). The three clones had variable amounts of untranslated DNA at the 3′ ends (GH2, 260 bp; GH17, 142 bp; and GH22, 166 bp). The complete nucleotide sequence of GH17 with the predicted protein sequence is shown in Fig. 1. The presumed initiation codon 36 bp downstream from the 5′ end is the first ATG in the ORF. The sequence also has a purine (adenine) in the −3 position (Kozak's rule), a prerequisite for an initiation codon (19). The initiation codon is followed by a hydrophobic sequence (predicted by hydropathy analysis [Fig. 2]) which is consistent with a signal peptide sequence. Two potential signal peptidase cleavage sites were identified by the method of von Heijne (37), which predicts cleavage after residues 20 and 24. The sequence also contains a predicted transmembrane helix from amino acid 2 to 28 (30). The 3′ noncoding region has a poly(A) tail of 14 bp. The ORF codes for a protein of 211 amino acids, with a predicted mass of 23.5 kDa and a calculated pI of 4.15. There are three potential N-glycosylation sites in the predicted amino acid sequence; these are located in the hydrophilic domains of the protein (Fig. 1). The only significant similarity between the GH17 sequence and the proteins present in the GenBank/EMBL sequence databases is that between its threonine-rich region and other threonine-rich sequences such as those of *Caldocellum saccharolyticum* cellulase (26), *Xenopus laevis* integumentary mucin (12), and a *Leishmania* surface antigen (28) (Fig. 3).

Southern blot analysis was performed to identify genomic fragments carrying the gene(s) encoding the recombinant clone GH17. When DNA was cut with *Eco*RI and *Pst*I and probed with the labeled cDNA insert of GH17, bands were detected at 4.9 and 5.5 kb, respectively (Fig. 4). The probe hybridized to two bands (8.5 and 5.0 kb) in *Sac*I-digested DNA. However, recombinant clone GH17 has an internal *Sac*I site. These results suggest a single location in the *H. capsulatum* genome for GH17.

Sensitivity and specificity of IgG antibodies to recombinant *H. capsulatum* proteins. The immunoreactivity of recombinant *H. capsulatum* proteins produced by clones GH2, -17, -22, and -23 was assessed by Western blotting with sera from patients with a variety of fungal infections. Most sera from histoplasmosis patients had easily visible antibody reactivity with all four recombinant proteins (Table 1 and Fig. 5a). The sensitivity of Western blotting with these clones for histoplasmosis sera ranged from 89 to 100% (Table 1 and Fig. 5a). None of these clones was recognized by sera from humans and animals infected with other fungi (Fig. 5b).

Expression of GH17. The cDNA insert of GH17 was expressed as histidine fusion in the pPROEX-1 protein expression system. Plasmid pPROEX-1 consists of a Trc promoter for high-level expression in *E. coli*, a prokaryotic ribosome binding site, and a six-His affinity tag for ease of purification. A fusion protein with an apparent mass of 32 kDa was evident by SDS-PAGE and immunoblotting (Fig. 6a). GH17-his failed to bind to a metal affinity column. Therefore, the fusion protein was purified from bacterial lysates by continuous-elution electrophoresis with a Bio-Rad Prep Cell. Western blotting was performed to select fractions of interest by immunoblotting with a human histoplasmosis serum pool (Fig. 6b). Three consecutive fractions containing the band of interest were selected, pooled, and dialyzed. This yielded a total of 500 μg of purified protein from approximately 700 ml of bacterial culture.

Pilot studies were carried out to test the purified GH17-his protein in an enzyme-linked immunosorbent assay (ELISA) format (data not shown). Unfortunately, ELISA based on the GH17-his protein was less sensitive and specific than the re-

```
A          GH17        38 PPTTTTTTTTTTPTPTPTSIIPITP 62
                          P +T T T T TPTPTPT     TP
       Cellulase      329 PTSTVTPTPTPTPTPTPTVTATPTP 353

B          GH17       38 PPTTTTTTTTTTPTPTPTSIIPITPIVPANKTIVLTTTIEP 78
                         PPTTTTTTTTTTT T T T+  PIT           TTT +P
           PSA       183 PPTTTTTTTTTTTTTTTTTTKPPITTATTTKPPTTTTTTTKP 223

C          GH17      22  TSGAAVDSCLLESNCPPPTTTTTTTTTTPTPTPTSIIPITPIVPANKTIVLTTTIEP 78
                         T+ A   +    +  P  TTTTTTTT   T TPT+  P T    A  T   TTT  P
           Xenopus   418 TTKATTTTPTTTTTTPTTTTTTTTTTKATTTTPTTTTPTTTTTKATTTTPTTTTTTP 474
```

FIG. 3. Comparison of the deduced amino acid sequence encoded by GH17 with threonine-rich regions of cellulase from a thermophilic bacterium, *C. saccharolyticum* (A), a *Leishmania* surface antigen (B), and integumentary mucin from *X. laevis* (C), performed with the National Center for Biotechnology Information BLAST analysis program. Identical residues are indicated by letters, and conserved residues are marked with a plus sign.

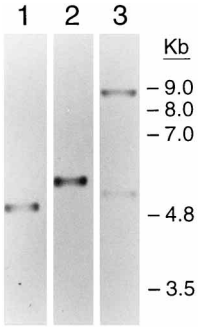

FIG. 4. Southern blot of genomic DNA of *H. capsulatum* probed with labeled cDNA insert from GH17. Genomic DNA was digested with *Eco*RI (lane 1), *Pst*I (lane 2), and *Sac*I (lane 3); electrophoresed on a 1% agarose gel; and transferred to a nylon membrane. The membrane was probed with the peroxidase-labeled cDNA insert of GH17 and washed under high-stringency conditions.

combinant immunoblot assay with the GH17 β-galactosidase fusion protein.

Immunoblot analysis of mouse antibodies to recombinant antigen. Sera from mice immunized with GH17-his bound to a 60-kDa native *H. capsulatum* yeast antigen as indicated by Western blotting (Fig. 7). This antigen was not recognized by preimmune mouse sera.

DISCUSSION

The goals of this study were to clone, characterize, and overexpress recombinant *H. capsulatum* antigens with immunodiagnostic potential. Recombinant clones that expressed *H. capsulatum*-specific antigens were identified by several cycles of differential immunoscreening, and the most immunoreactive and specific clone (GH17) was selected for more detailed studies. GH17 codes for the most promising recombinant diagnostic antigen for histoplasmosis that has been identified to date.

GH17 codes for a novel protein that corresponds to a 60-kDa native *H. capsulatum* antigen. There are three potential N-glycosylation sites (Asn-[Asn/Lys]-Thr) in the predicted amino acid sequence of GH17 (Fig. 1). Glycosylation at these sites could account for the difference between the predicted polypeptide mass of 23.5 kDa and the observed size of the native yeast protein recognized by the mouse antibodies to GH17 histidine fusion protein (60 kDa).

TABLE 1. Sensitivity[a] and specificity of immunoblot with recombinant *H. capsulatum* clones

Serum source	No. of reactive sera/no. tested for:			
	GH2	GH17	GH22	GH23
Histoplasmosis, human	18/18	18/18	16/18	16/18
Blastomycosis				
Dog	0/6	0/6	0/6	0/6
Rabbit	0/3	0/3	0/3	0/3
Human	0/5	0/5	0/5	0/5
Coccidioidomycosis, human	0/12	0/12	0/12	0/12
Candidiasis, human	0/5	0/5	0/5	0/5
Uninfected controls, human	0/12	0/12	0/12	0/12

[a] Immunoreactivity was assessed by immunoblot with β-galactosidase fusion proteins.

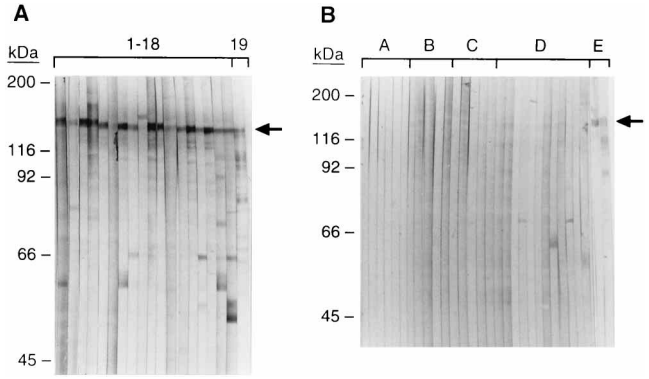

FIG. 5. (A) Representative immunoblot showing the immunoreactivity of the β-galactosidase fusion protein (arrow) of GH17. Bacterial cell lysates from cells infected with GH17 were separated by SDS-PAGE and electrophoretically transferred to nitrocellulose paper. The blot was developed with individual sera from patients with histoplasmosis (lanes 1 to 18). Sixteen of 18 sera had strong antibody reactivity with the fusion protein, and two were weakly reactive (lanes 6 and 9). Lane 19 was developed with a murine monoclonal antibody to β-galactosidase. (B) Demonstration of antigenic specificity of recombinant *H. capsulatum* GH17 β-galactosidase fusion protein by immunoblot analysis. Lanes were developed with sera from dogs infected with *B. dermatitidis* (n = 6) (A), patients infected with *B. dermatitidis* (n = 5) (B), patients infected with *Candida albicans* (n = 5) (C), and patients infected with *Coccidioides immitis* (n = 12) (D) and with a human histoplasmosis serum pool and anti-β-galactosidase antibody (E). The immunoreactive fusion protein band is indicated by an arrow.

The protein encoded by GH17 appears to be highly antigenic in humans with histoplasmosis. Chou-Fasman predictions based on the deduced amino acid sequence of GH17 (7) indicate that the protein is rich in potential B-cell epitopes. These predictions are based principally on the hydrophilic character and accessibility of highly charged and exposed polar residues that comprise the turns and alpha helices within the predicted GH17 protein (13, 21). Our results with human sera are consistent with these predictions. GH17 produced a 140-kDa fusion protein that was recognized in Western blots by 18 of 18 sera from patients with histoplasmosis.

Fungal antigen detection is often useful for the diagnosis of

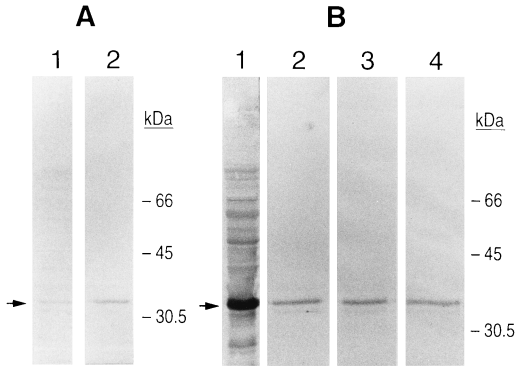

FIG. 6. (A) SDS-PAGE and immunoblot analysis of expression of GH17-his. SDS-PAGE (10% polyacrylamide) was loaded with *E. coli* extract before IPTG induction (lane 1) and after IPTG induction for 3 h (lane 2). The immunoblots were developed with human histoplasmosis serum pool (1:500), enzyme-labeled anti-human IgG secondary antibody, and substrate. (B) Immunoblot analysis of eluted fractions of GH17-his separated by preparative SDS-PAGE in the model 491 Bio-rad Prep Cell. Aliquots (10 μl each) from the prep cell fractions were separated by SDS-PAGE on 12% gels, immunoblotted, and developed as described for Fig. 6A. Lane 1, IPTG-induced *E. coli* extract (2 μl); lanes 2 to 4, positive fractions from the Prep Cell. The arrow indicates the 32-kDa GH17-his fusion protein.

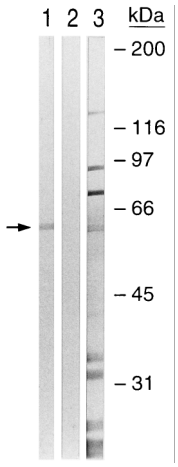

FIG. 7. Immunoblot analysis of *H. capsulatum* yeast antigen extract developed with mouse antibody to GH17-his (1:500) (lane 1), normal mouse serum (1:500) (lane 2), and mouse antibodies to *H. capsulatum* yeast extract (1:500) (lane 3). The arrow indicates 60-kDa native yeast antigen.

patients with disseminated histoplasmosis (39, 40), but this test is less sensitive in patients with localized disease, and cross-reactivity with other fungal infections has been reported (40, 41). Thus, antibody serology could be useful for the diagnosis of patients with early or localized infections. Unfortunately, existing antibody tests that employ native *H. capsulatum* antigens suffer from poor specificity (complement fixation and ELISA) or poor sensitivity (immunodiffusion). Several studies have demonstrated variable degrees of cross-reactivity in *H. capsulatum* antibody serology (15, 16). Sera from patients with confirmed blastomycosis, coccidioidomycosis, and candidiasis are sometimes reactive by indirect ELISA and by immunoblotting with *H. capsulatum* yeast and mycelial antigens (6a, 20). Thus, immunological cross-reactivity among fungi limits the value of antibody assays based on crude antigen mixtures. In contrast, the GH17 recombinant immunoblot assay appears to have excellent specificity for histoplasmosis. However, additional studies are needed to further define the sensitivity and clinical utility of antibody assays based on GH17.

ACKNOWLEDGMENTS

We thank Sharon Travis and Fanya Liftis for excellent technical assistance.

REFERENCES

1. **Ajello, J.** 1971. Coccidioidomycosis and histoplasmosis: a review of their epidemiology and geographical distribution. Mycopathol. Appl. **45:**221–230.
2. **Bauman, D. S., and C. D. Smith.** 1975. Comparison of immunodiffusion and complement fixation tests in the diagnosis of histoplasmosis. J. Clin. Microbiol. **2:**77–80.
3. **Buechner, H. A., J. H. Seabury, and C. C. Campbell.** 1973. The current status of serologic, immunologic, and skin tests in the diagnosis of pulmonary mycoses. Chest **63:**259–270.
4. **Chandler, J. W., T. K. Smith, and W. M. Newberry.** 1969. Immunology of mycoses. II. Characterization of the immunoglobulin and antibody responses in histoplasmosis. J. Infect. Dis. **119:**247–254.
5. **Chandrashekar, R., K. Masood, R. M. Alvarez, A. F. Ogunrinade, R. Lujan, F. Richards, and G. J. Weil.** 1991. Molecular cloning and characterization of recombinant parasite antigens for immunodiagnosis of onchocerciasis. J. Clin. Invest. **8:**1460–1466.
6. **Chandrashekar, R., K. C. Curtis, R. M. Ramzy, F. Liftis, B. W. Li, and G. J. Weil.** 1994. Molecular cloning of *Brugia malayi* antigens for diagnosis of lymphatic filariasis. Mol. Biochem. Parasitol. **64:**261–271.
6a. **Chandrashekar, R. et al.** Unpublished data.
7. **Chou, P. Y., and G. Fasman.** 1978. Prediction of the secondary structure of proteins from their amino acid sequence. Adv. Enzymol. Relat. Areas Mol. Biol. **47:**145–147.
8. **Edwards, P. Q., and E. L. Billings.** 1971. World-wide pattern of skin sensitivity to histoplasmin. Am. J. Trop. Med. Hyg. **20:**288–319.
9. **George, R. B., R. S. Lambert, M. J. Bruce, T. W. Pickering, and R. M. Wolcott.** 1981. Radioimmunoassay: a sensitive screening test for histoplasmosis and blastomycosis. Am. Rev. Respir. Dis. **124:**407–410.
10. **Goodwin, R., Jr., and R. M. Perez.** 1978. Histoplasmosis. Am. Rev. Respir. Dis. **117:**929–955.
11. **Goodwin, R. A., Jr., and R. M. Des Perez.** 1979. Histoplasmosis: state of the art. Am. Rev. Respir. Dis. **119:**247–254.
12. **Hauser, F., and W. Hoffman.** 1992. P-domains as shuffled cysteine-rich modules in integumentary mucin C.1 (FIM-C.1) from *Xenopus laevis*. Polydispersity and genetic polymorphism. J. Biol. Chem. **267:**24620–24624.
13. **Hopp, T. P.** 1986. Methods for identifying antigenic determinants and other interaction sites. J. Immunol. Methods **88:**1–18.
13a. **Hopp, T. P., and K. R. Woods.** 1981. Prediction of protein antigenic determinants from amino acid sequences. Proc. Natl. Acad. Sci. USA **78:**3824–3828.
14. **Kafatos, F. C., C. W. Jones, and A. Efstratiadis.** 1979. Determination of nucleic acid sequence homologies by a dot hybridization procedure. Nucleic Acids Res. **7:**1541–1552.
15. **Kaufman, L., and E. Reiss.** 1986. Serodiagnosis of fungal diseases, p. 446–466. *In* N. R. Rose, H. Friedman, and J. L. Fahey (ed.), Manual of clinical laboratory immunology, 3rd ed. American Society for Microbiology, Washington, D.C.
16. **Kaufman, L.** 1992. Laboratory methods for the diagnosis and confirmation of systemic mycoses. Clin. Infect. Dis. **14:**S23–S29.
17. **Keath, E. J., A. A. Painter, G. S. Kobayashi, and G. Medoff.** 1989. Variable expression of a yeast-phase-specific gene in *Histoplasma capsulatum* strains differing in thermotolerance and virulence. Infect. Immun. **57:**1384–1390.
18. **King, K.** 1982. Histoplasmosis in Australia. Aust. Med. J. **2:**550–551.
19. **Kozak, M.** 1986. Point mutations define a sequence flanking the AUG initiator codon that modulates translation by eukaryotic ribosomes. Cell **44:**283–292.
20. **Kumar, B. V., G. Medoff, G. S. Kobayashi, and W. L. Sieling.** 1985. Cross-reacting human and rabbit antibodies to antigens of *Histoplasma capsulatum*, *Candida albicans*, and *Saccharomyces cerevisiae*. Infect. Immun. **48:**806–812.
21. **Kyte, J., and R. F. Doolittle.** 1982. A simple method for displaying the hydropathic character of a protein. J. Mol. Biol. **157:**105–132.
22. **Laemmli, U. K.** 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature **227:**680–685.
23. **Lambert, R. S., and R. B. George.** 1987. Evaluation of enzyme immunoassay as a rapid screening test for histoplasmosis and blastomycosis. Am. Rev. Respir. Dis. **136:**316–319.
24. **Lobos, E., N. Weiss, M. Karam, H. R. Taylor, E. A. Ottesen, and T. B. Nutman.** 1991. An immunogenic *Onchocerca volvulus* antigen: a specific and early marker of infection. Science **51:**1603–1605.
25. **Lustigman, S., B. Brotman, T. Huima, and A. M. Prince.** 1991. Characterization of an *Onchocerca volvulus* cDNA clone encoding a genus-specific antigen present in infective larvae and adult worms. Mol. Biochem. Parasitol. **45:**65–76.
26. **Lüthi, E., N. B. Jasmat, R. A. Grayling, D. R. Love, and P. L. Bergquist.** 1991. Cloning, sequence analysis, and expression in *Escherichia coli* of a gene coding for a β-mannanase from the extremely thermophilic bacterium "*Caldocellum saccharolyticum*." Appl. Environ. Microbiol. **57:**694–700.
27. **Maniatis, T., E. F. Fritsch, and J. Sambrook.** 1982. Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
28. **Murray, P. J., and T. W. Spithill.** 1991. Variants of a *Leishmania* surface antigen derived from a multigenic family. J. Biol. Chem. **266:**24477–24484.
29. **Rao, A., R. Forgan-Smith, G. Ritchie, H. Sakellaris, S. Miller, and M. Arghyros.** 1988. A serendipitous isolate of *Histoplasma capsulatum*. Pathology **20:**191–193.
30. **Rao, M. J. K., and P. Argos.** 1986. A conformational preference parameter to predict helices in integral membrane proteins. Biochim. Biophys. Acta **869:**197–214.
31. **Reiss, E., H. Hutchinson, L. Pine, D. W. Ziegler, and L. Kaufman.** 1977. Solid-phase competitive-binding radioimmunoassay for detecting antibody to the M antigen of histoplasmin. J. Clin. Microbiol. **6:**598–604.
32. **Saiki, R. K., D. H. Gelfand, S. Stoffel, S. Scharf, R. Higuchi, G. T. Horn, K. B. Mullis, and H. A. Erlich.** 1988. Primer-directed enzymatic amplification of DNA with a thermostable DNA polymerase. Science **239:**489–491.
33. **Sanger, F., S. Nicklen, and A. R. Coulson.** 1977. DNA sequencing with chain terminating inhibitors. Proc. Natl. Acad. Sci. USA **74:**5463–5467.
34. **Schwarz, L.** 1981. Histoplasmosis. Praeger Science Press, New York, N.Y.
35. **Spitzer, E. D., E. J. Keith, S. J. Travis, A. A. Painter, G. S. Kobayashi, and G. Medoff.** 1990. Temperature-sensitive variants of *Histoplasma capsulatum*

isolated from patients with acquired immunodeficiency syndrome. J. Infect. Dis. **162:**258–261.
36. **Towbin, H., T. Staehelin, and J. Gordon.** 1979. Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. Proc. Natl. Acad. Sci. USA **76:**4350–4354.
37. **von Heijne, G.** 1986. A new method for predicting signal sequence cleavage sites. Nucleic Acids Res. **14:**4683–4690.
38. **Wheat, L. J., R. B. Kohler, M. L. V. French, J. Garten, A. White, and Z. Brahmi.** 1983. IgM and IgG histoplasmal antibody response in histoplasmosis. Am. Rev. Respir. Dis. **128:**65–70.
39. **Wheat, L. J., R. B. Kohler, and R. P. Tiwari.** 1986. Diagnosis of disseminated histoplasmosis by detection of *Histoplasma capsulatum* antigen in serum and urine specimens. N. Engl. J. Med. **314:**83–88.
40. **Wheat, L. J., P. Connolly-Stringfield, R. B. Kohler, P. T. Frame, and M. R. Gupta.** 1989. *Histoplasma capsulatum* polysaccharide antigen detection in diagnosis and management of disseminated histoplasmosis in patients with AIDS. Am. J. Med. **87:**396–400.
41. **Wheat, L. J., R. B. Kohler, R. P. Tiwari, M. Garten, and M. L. V. French.** 1989. Significance of *Histoplasma* antigen in the cerebrospinal fluid of patients with meningitis. Arch. Intern. Med. **149:**302–304.