JOURNAL OF CLINICAL MICROBIOLOGY, Dec. 1997, p. 3278–3283 Vol. 35, No. 12
0095-1137/97/$04.00+0

Use of Monoclonal Antibodies in Diagnosis of Paracoccidioidomycosis: New Strategies for Detection of Circulating Antigens

B. L. GÓMEZ,[1] J. I. FIGUEROA,[2] A. J. HAMILTON,[2] B. ORTIZ,[1]
M. A. ROBLEDO,[1] R. J. HAY,[2] AND A. RESTREPO[1]*

Corporación para Investigaciones Biológicas, Medellín, Colombia,[1] and St. John's Institute of Dermatology, Guy's Hospital, London SE19RT England[2]

Received 30 May 1997/Returned for modification 13 August 1997/Accepted 21 September 1997

The precise diagnosis of paracoccidioidomycosis, in most cases, is established by direct methods and indirect immunological tests. The latter method is reliant on the identification of the host's humoral responses, which are usually impaired or absent in patients with severe juvenile forms of the disease and in immunocompromised patients. Determining disease activity or assessing treatment responses by measuring antibody levels is difficult, since antibody titer may remain elevated or persist at stationary levels, even in the presence of clinical improvement. Consequently, there is a need for alternative tests aimed at the identification of circulating antigens. A modification of the standard hybridoma production method was used to raise a panel of murine monoclonal antibodies (MAbs) against the yeast form of *Paracoccidioides brasiliensis*. Of these, MAb P1B, directed against an 87-kDa determinant, was used to develop an inhibition ELISA (inh-ELISA) capable of detecting as little as 5.8 ng of circulating antigen per ml of serum. Sera from 46 patients with paracoccidioidomycosis or other mycoses and sera from healthy individuals were evaluated by the inh-ELISA; overall sensitivity was 80.4% (37 of 46 paracoccidioidomycosis patients tested positive), and specificity compared with that of normal controls from areas of endemicity was 81.4%. The inh-ELISA detected circulating antigen in 100% of patients with the acute form of paracoccidioidomycosis and in 83.3 and 60% of patients with the chronic multifocal and unifocal forms of paracoccidioidomycosis according to the patients' clinical presentation. These results indicate that the inh-ELISA with MAb P1B is effective in the detection of circulating antigen and that this test may be useful for monitoring responses to treatment and establishing disease prognoses.

Paracoccidioidomycosis (PCM), a disorder caused by the dimorphic fungus *Paracoccidioides brasiliensis*, is one of the most important systemic mycoses in Central and South America (1). The disease primarily involves the lungs and then disseminates to other organs and systems. Secondary lesions frequently appear in the mucous membranes, skin, lymph nodes, and adrenals. Two main clinical forms are recognized: the acute or subacute form (juvenile type) and the chronic form (adult type) (16). Both the clinical presentation and the course of the disease vary from patient to patient (1, 40), impeding prompt clinical diagnosis.

The definitive diagnosis of PCM can be accomplished only by laboratory procedures, mainly by the direct visualization of the fungus and its isolation in culture; however, the time required to isolate *P. brasiliensis* from clinical specimens represents a hindrance to rapid diagnosis (1). Since its introduction by Moses in 1916, the detection of serum antibodies against components of *P. brasiliensis* has been one of the main tools for diagnosis and for monitoring patients' responses to treatment (33). The most common serological tests used are complement fixation (11), immunodiffusion (4, 37), immunoenzymatic assays (2, 3, 6, 30, 42), counterimmunoelectrophoresis (9), and immunoblotting (5, 32). Unfortunately, there is extensive antigenic cross-reactivity between *P. brasiliensis* and other fungi (1, 8, 27, 30, 35), limiting the value of the tests that are currently employed. Cross-reactivity and the lack of antigen standardization due to variations in the isolates used as an antigen source (7, 10) create more difficulties. Data from many different studies make it evident that different tests with the same serum do not always give similar results. Consequently, it is advisable to employ more than one test for the diagnosis of PCM (39).

A different diagnostic approach is desirable for immunocompromised individuals. Antibody titer may be lower or absent in up to 50% of immunocompromised patients (18). The detection of *P. brasiliensis* circulating antigens in body fluids could facilitate the early diagnosis of the mycosis and confirm a preliminary diagnosis when antibody detection is nonconclusive. In addition, the monitoring of treatment response by measuring antigen levels would be an asset, since antibody titer may remain elevated even after apparent clinical remission (12, 34, 43). The detection of antigenemia has proved difficult in PCM patients, however, and it has been attempted by counterimmunoelectrophoresis (41), immunoelectrophoresis-immunodiffusion (20), passive hemagglutination inhibition, inverted linear immunoelectrophoresis (33), immunoradiometric assay (13), a competitive enzyme-linked immunosorbent assay (ELISA) (17), and immunoblotting (31). The majority of these reports indicate variations in sensitivity and specificity, probably due to the small number of patients evaluated. In this study we describe the development of an inhibition ELISA (inh-ELISA) for the detection of circulating antigen with a monoclonal antibody (MAb) produced by a modification of the standard hybridoma production technique. A representative number of serum samples from well-documented PCM patients was assessed together with sera from individuals with other mycoses and sera from healthy individuals living in areas of endemicity or areas of nonendemicity.

* Corresponding author. Mailing address: Laboratorio de Micologia, Corporación para Investigaciones Biológicas (CIB); Carrera 72A No. 78B141, AA 73-78 Medellín, Colombia. Phone: 57-4-4410855. Fax: 57-4-4415514. E-mail: cib@epm.net.co.

TABLE 1. Sources of serum and urine specimens tested

Group	No. of specimens[a]	
	Sera	Urine
PCM, all clinical forms	46	13
Acute	5	1
Chronic multifocal	31	8
Chronic unifocal	10	4
Other mycoses		
Histoplasmosis	10	12
Aspergillosis	10	
Cryptococcosis	10	
Sporotrichosis	9	
Tuberculosis	9	
Healthy persons from areas of endemicity	49	20
Healthy persons from the United Kingdom	20	
Total	163	45

[a] Each serum and urine sample is from a different patient.

MATERIALS AND METHODS

Clinical samples. A total of 46 patients with mycologically confirmed (direct KOH examination, isolation by culture, and positive serological tests) and active PCM were used in this study. One serum sample was taken from each patient at the time of diagnosis. In addition, 13 of these patients each provided a single urine sample. Samples were collected between January 1988 and December 1996 at the mycology laboratory, Corporación para Investigaciones Biológicas (CIB), Medellín, Colombia. Patients were classified according to their respective clinical presentations (16, 39) (Table 1). The mean age of patients with the acute juvenile form of the disease (including an AIDS patient) was 21 years, whereas the mean age of patients with the chronic forms of the disease was 48 years old. All patients but one were male. Serum samples ($n = 39$) and urine samples ($n = 12$) obtained at the time of the diagnosis from 39 different patients with other mycologically and/or serologically confirmed mycoses were also evaluated (Table 1). In addition, nine serum samples from patients with confirmed tuberculosis were studied. Negative controls included 49 normal human serum (NHS) samples and 20 urine samples from healthy volunteers with no history of lung disease, from areas where tuberculosis is endemic. In addition, 20 normal human serum samples were obtained from healthy individuals living in the United Kingdom.

Fungal strains and antigen preparation. Mycelial isolates from *P. brasiliensis* CIB 339 and *Histoplasma capsulatum* var. *capsulatum* isolates Hc 1980 and Hc 11265 were obtained from the culture collection of the CIB. Similarly, mycelial isolates of *Blastomyces dermatitidis* NCPF 4076 and *Sporothrix schenckii* Ss 17 were obtained from the National Collection of Pathogenic Fungi (Colindale, London, United Kingdom) and the St. John's Institute of Dermatology (London, United Kingdom), respectively. In addition, *Aspergillus fumigatus* isolate NCPF 2010 was used. Isolates of dimorphic fungi were transformed to the yeast phase, and antigen was produced as described elsewhere (14, 15, 22); *A. fumigatus* antigen was produced as previously detailed (23). The *P. brasiliensis* isolate was subcultured in McVeigh Morton media (38). A three-day-old culture was then transferred to a 500-ml flask containing 200 ml of McVeigh Morton liquid media, which was then placed in a gyratory shaker incubator at 35.5°C. Three days later, 10^7 cells were expanded into 200 ml of fresh McVeigh Morton liquid media and the culture was grown under the same conditions for up to 7 days. Yeast cultures were killed with thimerosal (0.02%, wt/vol) (Sigma, Poole, United Kingdom) and harvested by centrifugation at 3,000 × *g* for 30 min. The pellet was washed twice in phosphate-buffered saline (PBS; 0.01 M, pH 7.2). The pellet was then divided into two aliquots, one of which was treated with a mixture of protease inhibitors containing 1 mM phenylmethylsulfonyl fluoride (Sigma), 0.1 mM Pepstatin A (Sigma), 0.1 mM *N*-tosyl-L-phenylanine chloromethyl ketone (TPCK) (Sigma), 0.2 mM *N*-α-*p*-tosyl-L-lysine chloromethyl ketone (TLCK), and 1 mM EDTA (Sigma). Aliquots were homogenized with a bead beater (Biospec Products, Bartlesville, Okla.), and the homogenate was then centrifuged at 2,000 × *g* for 20 min at 4°C. The supernatant was recovered, and protein determinations were performed as described previously (14).

MAb production. On day 1, 10 female BALB/c mice (8 to 10 weeks old; Harlan Olac, Oxon, United Kingdom) were immunized intraperitoneally (i.p.) with 50 μg per 100 μl of an equal mixture of *H. capsulatum* var. *capsulatum* Hc 1890 and *B. dermatitidis* NCPF 4076 cytoplasmic yeast antigen (CYA) made up 1:1 in Freund's incomplete adjuvant (Difco, East Molesey, United Kingdom). Cyclophosphamide (Sigma) in sterile PBS was administered i.p. 10 minutes later at a dose of 50 mg/kg of body weight. The same dose was repeated 24 and 48 h later. Two control mice did not receive cyclophosphamide. Twelve days later, the mice received an i.p. inoculation with *P. brasiliensis* CIB B-339 CYA (50 μg/mouse, made up in a 1:1 ratio in Freund's incomplete adjuvant). This inoculation was repeated on day 20, and in order to determine which mouse had the highest differential polyclonal response on day 22, mice were tail bled and antibody responses to *P. brasiliensis* and *H. capsulatum* CYAs were quantified by ELISA, as previously described (14, 21). The chosen mouse received a further intravenous dose of 50 μg of *P. brasiliensis* CYA in 100 μl of sterile PBS, and its spleen was used for the fusion protocol 3 days later. Cells of the murine myeloma line Sp 2/0 were fused with spleen cells from the donor mouse as previously described (22). Hybridomas were screened by ELISA 7 days later, and colonies showing specificity against *P. brasiliensis* CYA were expanded onto 24-well plates and subcloned twice by limiting dilutions. Pristane (Sigma)-primed BALB/c mice were injected i.p. with 10^4 cells from each hybridoma line, and ascitic fluid was collected 7 to 10 days later. Culture supernatants of the different hybridoma lines were also collected and concentrated 100-fold by ammonium sulfate precipitation (24).

Characterization of MAbs. The specificity of MAbs was assessed by ELISA and Western blot assay, as described elsewhere (14, 22). MAbs were subclassed with a subclassing kit (Serotec, Kidlington, United Kingdom) as previously described (22).

inh-ELISA. A modification of the methods described by Freitas da Silva and Roque-Barreira (17) and Le Pape and Deunff (28) was used. An inh-ELISA was developed for both serum and urine samples. The diluting buffer used in the serum experiments consisted of a pool of NHS 1:10 in PBS 0.05%-Tween 20 (PBS-Tween), 20 mM $MgCl_2$, and 1% bovine serum albumin (Sigma Chemical Co., St. Louis, Mo.). The diluting buffer for experiments with urine was the same but contained a pool of normal human urine (NHU) instead of NHS. MAb P1B concentrated culture supernatant was used at a constant concentration of 1:70,000, and all samples were tested 1:2 in diluting buffer.

Inhibition plate. An inhibition standard curve was constructed by adding different concentrations of *P. brasiliensis* CIB 339 CYA (from 4 ng to 60 μg per ml) to 100 μl of pooled NHS (or NHU) and then adding 100 μl of the standardized dilution of MAb P1B. NHS or NHU made up 1:2 in diluting buffer was used as a negative control. All the standards, samples, and controls were tested in duplicate. Samples were plated on 96-well round microtiter plates (Nunc A/S, Kamstrup, Denmark) previously blocked by incubation with 200 μl of 5% bovine serum albumin per well made up in PBS-Tween, for 2 h at 37°C. Plates were mixed in a shaker for 30 min at room temperature and then incubated overnight at 4°C.

Reaction plate. Polystyrene plates (Maxisorp; Nunc A/S) were coated with 0.5 μg of *P. brasiliensis* CIB 339 CYA in 0.06 M carbonate buffer (pH 9.6) per well. The plates were left for 30 min at room temperature and then incubated overnight at 4°C. After incubation, the plates were washed three times in PBS-Tween and blocked by incubation with 200 μl of 1% bovine serum albumin in PBS per well for 1 h at 37°C; after three more washes, 100 μl from each well in the inhibition plate (containing a mixture of the MAb-circulating antigen complexes and free MAb) was transferred to the respective wells in the reaction plate and allowed to stand for 2 h at 37°C. After being washed as described above, 100 μl of goat anti-mouse immunoglobulin G P_0 (Jackson, West Grove, Pa.) was added and the plates were incubated for another hour at 37°C. After further washings, the reaction was developed with a solution of *o*-phenylenediamine (0.2 mg/ml; Sigma) and 0.005% H_2O_2. The reaction was stopped with 4 N H_2SO_4 after 15 min of incubation in darkness. Optical densities (ODs) were measured at 490 nm with an ELISA plate reader (Microplate Readers 450/550; Bio-Rad, Richmond, Calif.). The OD at 490 nm was then plotted on a standard curve constructed from the data derived from MAb titration with NHS containing known quantities of *P. brasiliensis* CYA, as described above. The degree of inhibition in the binding of the MAb was shown to be reciprocal to the concentration of circulating antigen in the sample.

The cutoff point was established as the upper limit of the 90% least significant difference (LSD) confidence interval of the OD values obtained with the negative controls (NHS or NHU).

Statistical analysis. The inhibition standard curves were performed in duplicate for at least four independent assays. A regression model was constructed with the reciprocal values of antigen concentrations and the OD values obtained. Comparisons were done by one-way analysis of variance. Intergroup comparisons were performed by the multiple-range test with the LSD (90%). Statistical analysis was performed with Statgraphics Plus (release 1996; Statgraphics Corp., Rockville, Md.). Specificity and sensitivity were analyzed by the method of Gallen and Gambino (19).

RESULTS

MAb production. After serial subcloning, a panel of different hybridoma lines reactive to *P. brasiliensis* antigen was produced. Figures 1 and 2 illustrate the reactivity of MAb P1B culture supernatant against different fungal antigens. MAb P1B belongs to the immunoglobulin G G_1 subclass (data not shown) and recognizes an antigenic determinant of *P. brasiliensis* by ELISA (Fig. 1) with a relative molecular mass of 87

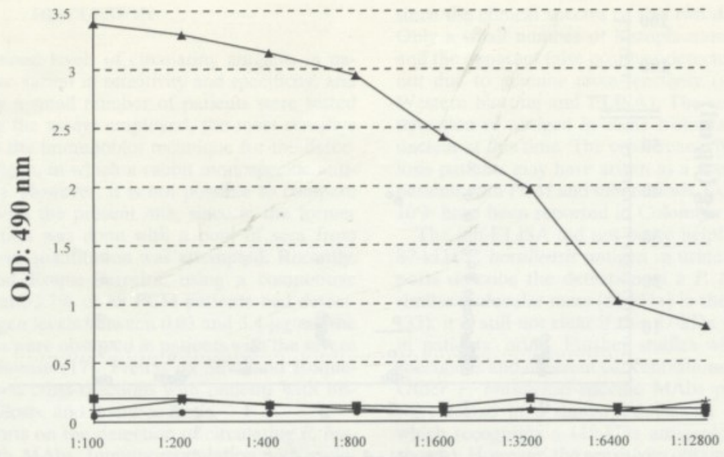

FIG. 1. Differential reactivities of increasing dilutions of MAb P1B culture supernatant in an ELISA to CYAs of various dimorphic fungi. *P. brasiliensis* CIB 339 CYA (▲), *H. capsulatum* CIB Hc 1980 CYA (■), *B. dermatitidis* NCFP 4076 CYA (●), and *S. schenckii* Ss 17 CYA (*).

kDa by Western blotting (Fig. 2). This MAb showed no recognition of CYAs from *H. capsulatum*, *S. schenckii*, or *B. dermatitidis*. However, by Western blotting, it recognized two poorly defined smears of between 32 and 42 and 45 and 80 kDa in *A. fumigatus* antigen. MAb P1B was used to develop an inh-ELISA test.

Detection of *P. brasiliensis* antigenemia by the inh-ELISA. Figure 3 represents the standard inhibition curve constructed with known quantities of *P. brasiliensis* CYA. The correlation coefficient of the curve (r) was 0.9611. This curve was used to determine the concentration of *P. brasiliensis* antigen in each sample tested. The sensitivity of the inh-ELISA ranged from 0.0058 to 60 μg of antigen per ml of serum. The cutoff point was fixed as the upper limit of the 90% confidence interval of the negative control readings. Positive results had an antigen concentration greater than 3.3 μg/ml.

Overall, 80.4% of the 46 PCM serum samples had detectable levels of circulating antigen above the cutoff point (Fig. 4), with a mean antigen concentration of 15.0 μg/ml. Table 2 shows the results obtained when patients' sera were separated according to the different clinical forms. Circulating antigen was detect-

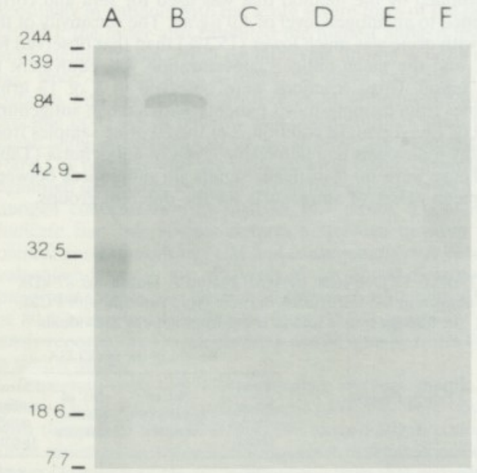

FIG. 2. Reactivity of culture supernatant of MAb P1B by Western blot assay to antigen preparations from different fungi. Lane A, molecular size markers; lane B, *P. brasiliensis* CIB 339 CYA; lane C, *H. capsulatum* CIB Hc 1980 CYA; lane D, *B. dermatitidis* NCPF 4076 CYA; lane E, *S. schenckii* Ss 17 CYA; and lane F, cytoplasmic antigen of *A. fumigatus* NCPF 2010, all at a concentration of 5 μg per lane.

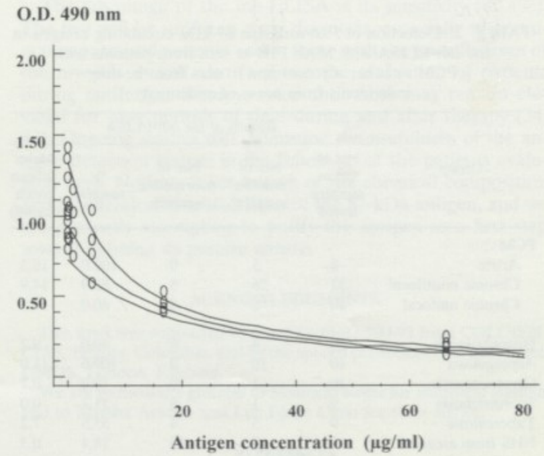

FIG. 3. Standard inhibition curve with MAb P1B. Regression model of absorbances against known concentrations of *P. brasiliensis* CIB 339 CYA. The correlation coefficient was 0.9611.

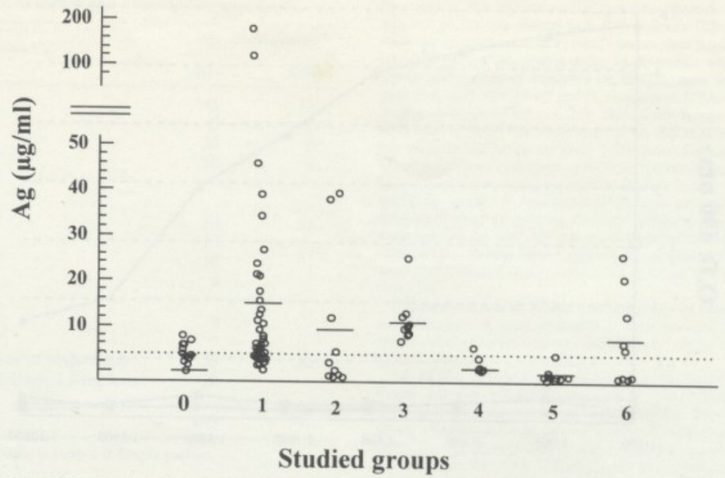

FIG. 4. inh-ELISA with MAb P1B for the detection of circulating antigen in sera from patients with PCM or other mycoses and sera from healthy controls from areas of endemicity. Groups studied: 0, NHS from areas of endemicity ($n = 49$); 1, PCM ($n = 46$); 2, histoplasmosis ($n = 10$); 3, aspergillosis ($n = 10$); 4, cryptococcosis ($n = 10$); 5, sporotrichosis ($n = 9$); 6, tuberculosis ($n = 9$). The dotted line represents the cutoff point equivalent to an antigen (Ag) concentration of 3.3 μg/ml. Solid lines represent the mean antigen concentration for each group. Data for normal human controls from the United Kingdom (20) are not shown; all sera were negative.

able in all patients with the acute form of the disease (mean, 16.25 μg/ml), in 83.8% of those patients with the chronic multifocal form of the disease (mean, 14.92 μg/ml), and in 60% of the patients with the chronic unifocal form of the disease (mean, 14.7 μg/ml).

Cross-reactions were observed in 19 of 48 heterologous serum samples, specifically with aspergillosis (10 of 10), histoplasmosis (4 of 10), and tuberculosis (5 of 9) serum samples. False-positive reactions were observed in 18.4% (9 of 49) of the NHS samples from patients who lived in areas where PCM is endemic, although the mean antigen concentration for this group was very low (0.3 μg/ml). The 20 serum samples from healthy individuals residing in the United Kingdom did not contain any detectable antigen (data not shown). Comparison (analysis of variance) of the mean antigen concentration observed in controls (NHS from patients living in areas where PCM is endemic and non-PCM sera) and in patients with the different forms of PCM showed statistically significant differences ($P < 0.0146$). There were also statistically significant differences (multiple-range test) when the antigen levels of the different clinical forms of PCM were compared with those in sera from healthy controls and those in sera from patients with sporotrichosis and cryptococcosis but not with those in the remaining heterologous sera.

TABLE 2. Detection of *P. brasiliensis* 87-kDa circulating antigen in the inh-ELISA with MAb P1B in sera from patients with PCM or other mycoses and in sera from healthy individuals from areas of endemicity

Group	Results of the inh-ELISA				
	No. of serum samples tested	No. of reactive samples[a]	No. of nonreactive samples	% reactive	Mean antigen concn (μg/ml)
PCM					
Acute	5	5	0	100.0	16.3
Chronic multifocal	31	26	5	83.9	14.9
Chronic unifocal	10	6	4	60.0	14.7
Histoplasmosis	10	4	6	40.0	9.2
Aspergillosis	10	10	0	100.0	11.0
Cryptococcosis	10	1	9	10.0	0.7
Sporotrichosis	9	1	8	11.1	0.0
Tuberculosis	9	5	4	55.5	7.2
NHS from areas of endemicity	49	9	40	18.4	0.3

[a] A sample was considered reactive if the mean antigen concentration was >3.3 μg/ml.

Detection of *P. brasiliensis* antigenuria by the inh-ELISA. A standard inhibition curve standard was constructed ($r = 0.9894$; data not shown). The cutoff level for positivity in this test was determined by the method that was used for sera and corresponded to an antigen level of 4.0 μg/ml. The sensitivity of the test with urine was much lower (15.3%) than that observed for sera, and the mean antigen concentration was equivalent to 8.37 μg/ml. Cross-reactions were observed in 3 of 12 urine samples from histoplasmosis patients, with a mean antigenuria level of 1.63 μg/ml. In addition, 2 of the 20 urine samples from healthy individuals had detectable levels of antigenuria (Table 3). There were no statistically significant differences between the mean values of antigenuria for the different groups.

TABLE 3. Detection by MAb P1B of *P. brasiliensis* 87-kDa antigen in the inh-ELISA in urine from patients with PCM or histoplasmosis and in urine from healthy individuals

Patient Group	Results of the inh-ELISA			
	Total no. of samples tested	No. of nonreactive samples[a]	No. of reactive samples	Mean antigen concn (μg/ml)
PCM	13	11	2	8.73
Histoplasmosis	12	9	3	1.63
Normal controls from regions of endemicity	20	18	2	0.01

[a] A sample was considered nonreactive if the mean antigen concentration was <4.0 μg/ml.

DISCUSSION

Previously determined levels of circulating antigens in patients with PCM have varied in sensitivity and specificity, and in most reports only a small number of patients were tested (13, 20, 42). Among the assays employed, the most sensitive described thus far is the immunoblot technique for the detection of the gp43 antigen, in which a rabbit monospecific antiserum was used (31). However, it is not possible to compare that study directly with the present one, since in the former study antigen detection was done with a pool of sera from PCM patients, and no quantitation was attempted. Recently, Freitas da Silva and Roque-Barreira, using a competitive ELISA, reported that 33.7% of 88 PCM patients had detectable circulating antigen levels between 0.03 and 3.4 μg/ml; the highest antigen levels were observed in patients with the severe acute form of the disease (17). Freitas da Silva and Roque-Barreira also reported cross-reactions with patients with histoplasmosis, aspergillosis, and cryptococcosis.

There are no reports on the detection of circulating *P. brasiliensis* antigens with MAbs. Immunomodulation with cyclophosphamide has previously been incorporated in immunization protocols to produce species-specific MAbs against different fungi (14, 21). The application of this methodology in the present study resulted in the production of MAb P1B, which recognizes an 87-kDa antigenic determinant of *P. brasiliensis*. MAb P1B appears to be species-specific by ELISA and Western blotting in tests against a panel of CYAs from different dimorphic fungi; however, MAb P1B recognizes *A. fumigatus* antigen by Western blotting. Based on the pattern of recognition on the Western blot, the composition of this cross-reactive *A. fumigatus* determinant appears to differ from that of the *P. brasiliensis* 87-kDa determinant, although there is no information yet about the precise nature of the antigens that were recognized in either case.

MAb P1B has been used to develop an inh-ELISA, which, as described in the present study, can detect antigen concentrations as low as 0.0058 μg/ml in serum. The overall sensitivity of the test among the 46 PCM patients was 80.4%, with a higher sensitivity in patients with the acute severe form of the disease (100%); the latter also exhibited the highest antigen levels of antigenemia (mean, 16.25 μg/ml). This finding is not surprising, since antigen load is higher in these patients. Among patients with the chronic forms of PCM, antigenemia was detectable in 83.6% of patients with the multifocal form, with a mean antigen level of 14.92 μg/ml; surprisingly, more than half (60%) of patients with the unifocal form (slight involvement) exhibited detectable levels of circulating antigen, with a mean antigen concentration as high as 14.7 μg/ml. These results indicate that this antigen detection test can be used in all clinical presentations of PCM and consequently may become a valuable adjunct in the diagnosis of this fungal disorder. It is important to note that the overall sensitivity of the inh-ELISA, at 80.4%, was much higher than the 33.7% sensitivity previously reported by Freitas da Silva and Roque-Barreira (17) in a similar study.

Important cross-reactions with other mycoses, mainly aspergillosis (10 of 10) and histoplasmosis (4 of 10), were observed with this inh-ELISA. However, the mean antigen concentrations observed in these heterologous sera were considerably lower (11 and 9.17 μg/ml, respectively) than that recorded for PCM patients (15.01 μg/ml). The reactivity of the aspergillosis patients can be accounted for in terms of genuine cross-reactivity, since we have shown that MAb P1B recognized *A. fumigatus* antigen; we believe that this cross-reactivity will not hinder the use of this diagnostic tool in the detection of PCM, since the clinical spectra of this two diseases seldom overlap. Only a small number of histoplasmosis patients were tested, and the apparent false positives detected in this population are not due to genuine cross-reactivity (according to data from Western blotting and ELISA). The reasons for the apparent detection of antigen in some histoplasmosis patients remain unclear at this time. The cross-reactivity observed for tuberculosis patients may have arisen as a result of the coinfection of patients with PCM and tuberculosis. Coinfection levels of up to 10% have been reported in Colombia (1).

The inh-ELISA did not prove helpful in the search for the 87-kDa *P. brasiliensis* antigen in urine. Although previous reports describe the detection of a *P. brasiliensis* antigen of a similar molecular mass (88 kDa) in the urine of PCM patients (33), it is still not clear if this 87-kDa determinant is excreted in patients' urine. Further studies with a larger number of specimens and different concentrations of samples are needed. Other *P. brasiliensis*-specific MAbs produced in this fusion were tested in a similar inh-ELISA system (e.g., MAb P8, which recognizes a 119-kDa antigenic determinant, data not shown). However, the sensitivity obtained with this reagent was too low (20%). As has been suggested for the diagnosis of other mycoses (29), it may be necessary to use a cocktail of MAbs directed against different specific antigenic determinants of the same fungus in order to increase detection rates. Such MAbs would have to be species-specific themselves to negate problems associated with cross-reactivity.

The major advantage of MAbs is their unique specificity (25). However, as MAbs have been used more widely, it has become apparent that intrinsic cross-reactions can occur, probably due to different species' having some common antigenic determinants or to similar determinants of related chemical structures; the coincidental expression of a determinant's shape has also been implicated (25). This type of cross-reactivity indicates that multispecificity may be found with both monoclonal (26) and polyclonal antibodies (44), albeit with a more limited range in the former. Ongoing studies concerning the molecular and immunological characterization of the 87-kDa determinant, which might be shared by *A. fumigatus*, will help to elucidate this problem and, we hope, to overcome it, as shown previously for the *P. brasiliensis* gp43 antigen determinant (36, 42).

The advantage of the inh-ELISA is its sensitivity (80.4%). The test will be useful in early diagnosis, especially in immunocompromised patients and in those with the juvenile form of the mycosis. It will also facilitate the evaluation of patients during antifungal therapy, since antibodies may remain elevated for long periods of time during and after therapy (34, 43). Ongoing studies will determine the usefulness of the antigen detection system in the follow-up of the patients evaluated here. Nothing is yet known of the chemical composition and biochemical characteristics of the 87-kDa antigen, and we are presently attempting to purify the antigen as a first step toward defining its precise nature.

ACKNOWLEDGMENTS

This work was supported by grant 22-13-02-500-93 from COLCIENCIAS, Bogota, Colombia, and by the special trustees of Guy's Hospital, UMDS, London, England.

We are particularly grateful to Mauricio Rojas for statistical analysis and to Myrtha Arango and Luz Elena Cano for their advice.

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