

Monoclonal Antibodies Against the 43,000 Da Glycoprotein from *Paracoccidioides brasiliensis* Modulate Laminin-Mediated Fungal Adhesion to Epithelial Cells and Pathogenesis

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ABSTRACT

The surface glycoprotein gp43, a highly immunogenic component of *Paracoccidioides brasiliensis*, is used in the serodiagnosis of paracoccidioidomycosis (PCM) and has recently been shown to specifically bind the extracellular matrix protein laminin. Binding to laminin induces the increased adhesion of the fungus to epithelial cells; a hamster testicle infection model has shown that the gp43-dependent binding of fungal cells to laminin enhances their pathogenicity in vivo. We report on the production and characterization of 12 monoclonal antibodies against the gp43 that recognize peptide sequences in the molecule detecting at least three different epitopes as well as different isoforms of this antigen. MAbs interfered in the fungal pathogenicity in vivo either by inhibiting or enhancing granuloma formation and tissue destruction. Results suggest that *P. brasiliensis* propagules may start infection in man by strongly adhering to human lung cells. Thus, laminin-mediated fungal adhesion to human lung carcinoma (A549) cells was much more intense than to Madin-Darby canine kidney cells (MDCK), indicating differences in binding affinity. Subsequent growth of fungi bound to the lung cells could induce the granulomatous inflammatory reaction characteristic of PCM. Both steps are greatly stimulated by laminin binding in infective cells expressing gp43.

INTRODUCTION

Paracoccidioides brasiliensis is a dimorphic fungus that causes paracoccidioidomycosis in man, an endemic disease prevalent in the subtropical regions of Latin America. The infection probably starts by inhalation of fungal propagules that differentiate into the infective yeast forms. Yeasts multiply in the lung, inducing the host response characterized by a primary complex or compact granuloma. Pulmonary or lymph node granuloma may remain dormant for years or else progress to overt disease.⁽¹⁻³⁾ Disseminated paracoccidioidomycosis is potentially lethal in untreated and/or immunosuppressed individuals.

The surface glycoprotein gp43 is the main diagnostic antigen of *P. brasiliensis*⁽⁴⁾ that can be isolated from different fungal extracts and is biochemically characterized as a high-mannose Concanavalin A-binding glycoprotein with an apparent

molecular weight of 43,000,⁽⁵⁻⁷⁾ with isoforms of pIs in the 5.8 to 8.5 range.⁽⁸⁾ Gp43 is recognized by all sera from infected patients using different serologic assays,^(9,10) and can be used to monitor the progression or cure of the mycosis.^(1,4) Monoclonal antibodies (MAbs) against peptide sequences of the molecule have been obtained previously⁽⁶⁾ and shown to react with glycosylated and deglycosylated forms of the gp43.

In addition to being the main diagnostic molecule of paracoccidioidomycosis the gp43 was also shown to express immunodominant epitopes eliciting T-cell-dependent delayed hypersensitivity reaction.⁽¹¹⁾ A T-CD4⁺ lymphocyte proliferation was observed in mice and sensitized humans in the presence of purified gp43.⁽¹²⁾ Moreover, we have shown previously that the gp43 specifically binds the extracellular matrix protein laminin with high affinity and that this binding could influence the fungal adhesion to epithelial cells.⁽¹³⁾

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Laminin is a 850 kDa glycoprotein of basement membranes that mediates cell adhesion, differentiation, shape, and motility.^(14,15) Its specific recognition was correlated to the invasiveness and pathogenesis of different infectious agents.⁽¹⁶⁾ Accordingly, we have showed that laminin enhances *P. brasiliensis* pathogenicity as determined by increased spreading and severity of the granulomatous disease in the hamster testicle.⁽¹³⁾ To further characterize the importance of the gp43 as a virulence factor we presently report on the characterization of 12 MAbs that recognize different epitopes and isoforms of the gp43 and are able to modulate the experimental infection by *P. brasiliensis*. We also show that the laminin-mediated adhesion of *P. brasiliensis* to human lung epithelial cells is much more intense than that to Madin-Darby canine kidney (MDCK) cells and can be inhibited by antibodies raised against a laminin receptor molecule.

MATERIALS AND METHODS

Fungal strains

P. brasiliensis strain B-339, provided by A. Restrepo, Corporación para Investigaciones Biológicas, Medellín, Colombia, and strain 18, by V. Calich, Universidade de São Paulo, were maintained by frequent subculturing on Sabouraud glucose agar (Difco BRL Products, Gaithersburg, MD). Yeast forms were grown in this medium at 35° C and were subcultured every third day. All experiments described below were performed with both fungal strains.

Cell lines

A 549 human lung carcinoma cell line,⁽¹⁷⁾ provided by Radovan Borojevic, Institute of Chemistry, and Madin-Darby canine kidney cells (MDCK) provided by Wanderley de Souza, Institute of Biophysics, both from Federal University of Rio de Janeiro, were propagated in RPMI 1640 (Sigma, St. Louis, MO) with 10% fetal calf serum (Cultilab, São Paulo). For adherence assays, cells were plated in 96-well tissue culture plates and grown to confluence at 37° C in an atmosphere of 5% CO₂ and 95% humidity. For immunofluorescence studies both cell lines were grown in chamber slides (Nunc) until confluence and fixed in cold acetone. Fixed cells were then treated subsequently with laminin anti-laminin rabbit polyclonal serum and revealed with goat anti-rabbit IgG-FITC (BioRad Labs., Hercules, CA). In another set of experiments, monolayers were treated with the anti-laminin polyclonal serum and revealed with anti-rabbit IgG-FITC. Reactions were visualized in a Nikon Epi-fluorescence microscope.

Preparation of fungal antigens

P. brasiliensis was grown on Sabouraud glucose agar at 35° C for 3 days and transferred to 50 ml of TOM medium as previously described.⁽⁵⁾ This pre-inoculum was cultivated for 3 days and then transferred into Fernbach flasks containing 500 ml of the same medium. After 7 days of incubation at 37° C cells were killed by the addition of 0.2 g per liter of thimerosal, the suspension was filtered in paper, and the supernatant fluid was collected by centrifugation. After dialysis against distilled water, the resulting material represented the crude exoantigen.

In order to purify gp43 the exoantigen was fractionated by affinity chromatography in columns of protein A-purified rabbit anti-gp43 IgG, coupled to Affi-Gel 10 (BioRad), as previously described.⁽⁶⁾ After obtaining anti-gp43 MAbs, similar affinity columns were made with MAb 17c.

Production of MAbs

MAbs were produced in our laboratory by Puccia and Travassos.⁽⁶⁾ Six-week-old BALB/c mice were immunized every 3 weeks subcutaneously with 50 µg of gp43 in PBS, incorporated in Freund's complete adjuvant for the first injection, and in incomplete Freund's adjuvant for the subsequent ones. Injections were always made in four different sites, in the axillary and inguinal regions, in final volumes of 100 µl per site. Before each immunization mice were bled through the ocular plexus, the serum was separated by centrifugation and stored at -20° C. Final immunization (50 µg of gp43 in 100 µl of PBS, intravenously) was made 2 days preceding cell fusion, according to Lopes and Alves.⁽¹⁸⁾ Screening of positive colonies was made by an enzyme immunoassay (EIA), as described later. After cloning by limiting dilution and expanding positive clones, large amounts of antibodies were obtained by producing ascites in BALB/c mice previously primed with Pristane (Sigma). MAbs were purified from both culture supernatants and ascites fluids by affinity chromatography in a Protein A column. Immunoglobulin isotyping was performed with the Clone Selector mouse monoclonal antibody screening kit (BioRad), according to the manufacturer's instruction.

Antibody screening by enzyme immunoassay (EIA)

EIA was performed as described elsewhere.⁽⁷⁾ Briefly, polyvinyl microplates (Costar) were coated with 50 µl of a 2 µg/ml solution of purified gp43 in PBS during a hour at room temperature. After blocking free sites with PBS containing 1% (v/v) bovine serum albumin (Sigma) (PBS-BSA), 50 µl of culture supernatant or purified MAbs were added to each well. After 1 hour of incubation at 37° C, wells were thoroughly washed with PBS containing 0.5% gelatin (Difco) and 0.05% Tween 20 (Sigma) (PTG) and treated with affinity-purified peroxidase-conjugated goat anti-mouse Ig (BioRad) for 1 hour at 37° C. This was followed by three washes with PTG. Reactions were developed by the addition of *o*-phenylenediamine (OPD) in 0.1 M acetate-phosphate buffer, pH 5.8, terminated with 4 N sulfuric acid, and read in a Titertek Multiscan EIA reader at 492 nm.

Inhibition assays

Purified MAbs 17c and 21f were labeled with horseradish peroxidase (Sigma, Type VI) according to the method described by Avrameas et al.⁽¹⁹⁾ Polyvinyl plates were coated with purified gp43 as described previously and treated with mixtures of labeled MAb plus different concentrations of each of the unlabeled MAb. After this incubation period, reactions proceeded as described. Inhibition by carbohydrates was tested with previous incubation of MAbs with 25, 50, and 100 mM solutions of mannose, galactose, N-acetylglucosamine, α -methyl-D-mannopyranoside, β -methyl-D-galactopyranoside, and glucose (Sigma). All reactions were made in triplicate.

Capture assays

Wells of polyvinyl microplates were coated with 10 $\mu\text{g/ml}$ solutions of purified anti-gp43 MAbs or with MAb 6.C4⁽²⁰⁾ and blocked with PBS containing 1% BSA. Purified gp43, previously labeled with ¹²⁵I by the IodoGen method⁽²¹⁾ to a specific activity of 3 μCi per μg was added at a concentration of 10 ng to each well. After 4 hours of incubation at room temperature and three washes with PTG, wells were cut and counted in a Packard Gamma Counter. All reactions were made in triplicate, and the results are expressed as percentages of captured antigen from the total labeled gp43 added in the system.

Immunoprecipitation assays

¹²⁵I-labeled gp43 was mixed with solutions of 2 $\mu\text{g/ml}$ of each purified MAb and incubated for 2 hours at 37°C. *Staphylococcus aureus*, Cowan I strain, prepared as previously described,⁽²²⁾ was added (50 μl of a 10% suspension) and incubated at room temperature for 30 minutes. The bacterial pellet was washed twice by centrifugation with PBS containing 1% Triton X-100, 0.1% BSA, and 0.02% sodium azide (v/v) and counted in a Packard Gamma counter. The unrelated MAb 6.C4⁽²⁰⁾ and rabbit anti-gp43 serum were used as negative and positive controls, respectively. Immunoprecipitation of the radioactive antigen with rabbit anti-gp43 serum at 1:200 dilution, was taken as 100%. The role of anti-gp43 MAbs in the binding of gp43 to ¹²⁵I-laminin (specific activity 7 $\mu\text{Ci}/\mu\text{g}$) was evaluated in a similar immunoprecipitation assay. Briefly, 10 $\mu\text{g/ml}$ of MAbs 24a, 8a, and 17c or rabbit anti-gp43 polyclonal antibodies were preincubated with 2 $\mu\text{g/ml}$ of affinity purified gp43 for 1 hour; then ¹²⁵I-laminin was added and the whole system was incubated for 1 hour, at 37° C. The pellet was washed and counted as described before.

SDS-PAGE and Immunoblot analysis

SDS-PAGE was performed on vertical slab gels of 10% or 12.5% acrylamide, always under reducing conditions, according to Laemmli.⁽²³⁾ Immunoblots were performed as described elsewhere.⁽²⁴⁾ Electrophoresed material was transferred to nitrocellulose membranes in a BioRad Trans-blot system at 100 V for 2 hours. Species-specific, affinity-purified peroxidase conjugates (BioRad) were used throughout.

Enzymatic proteolysis of gp43

Partial proteolysis of purified gp43 (100 μg) from *P. brasiliensis* B-339 was performed with papain (400 ng; Sigma), in phosphate buffer 100 mM, pH 6.5. After 30 minutes of incubation the reaction was terminated by boiling the sample in SDS-PAGE buffer. The product of reaction was analyzed in 12.5% acrylamide gels. Immunoblots were carried out as described.⁽²⁴⁾

Enzymatic deglycosylation of gp43

Deglycosylation of affinity-purified gp43 (10 μg) from *P. brasiliensis* B-339 was performed with recombinant PGNase (New England BioLabs) following the manufacturer's instructions. After 24 hours of incubation the reaction products were analyzed in 10% polyacrylamide gels (SDS-PAGE) and immunoblotted as described before.

Isoelectric focusing

Isoelectric focusing (IEF) was performed with the Pharmacia LKB PhastSystem apparatus. One μg of affinity purified gp43 from *P. brasiliensis* B-339 was submitted to IEF analysis using PhastGel IEF in a pH range of 3 to 9 (Pharmacia, Sweden), according to the manufacturer's instructions. Standard markers for isoelectric point determination of proteins in the same pH range (Pharmacia, Sweden) were used. Two gels were prepared. One of them was silver stained (PhastGel Silver Kit, Pharmacia, Sweden), and the other was immunoblotted with the PhastTransfer Semi-Dry Transfer Kit (Pharmacia, Sweden).

Laminin-mediated adhesion of *P. brasiliensis* to epithelial cell lines

Yeast forms of *P. brasiliensis* strain 18 were labeled with ¹²⁵I by the IodoGen method as described elsewhere⁽¹³⁾ and added to confluent monolayers of MDCK or A 549 cells, in the presence or absence of 20 $\mu\text{g/ml}$ of mouse laminin. Cells were previously grown in 96-well polystyrene plates (Costar) and washed with PBS. For inhibition assays labeled yeast forms (3×10^4 cells) were preincubated with 50 $\mu\text{g/ml}$ of anti-gp43 MAbs 40.D7, 17c, 24a, and 8a for 2 hours. Polyclonal rabbit sera anti-gp43 or anti-laminin, both diluted 1:100 were used as controls. Murine laminin at 20 $\mu\text{g/ml}$ was then added to the system. After 1 hour, 100 μl of treated yeast cells was added onto the cell monolayer. After 2 hours of incubation, nonadherent yeast cells were removed by aspiration and the monolayers washed twice with PBS. Estimation of bound yeast cells was performed after alkaline hydrolysis by counting the radioactivity in the lysate in a Packard Gamma Counter.

Hamster infections

Three-month-old male hamsters, obtained from the Animal Farm, Botucatu, São Paulo State University, divided into groups of five animals each, were injected in the left testicle with 10⁶ viable forms of *P. brasiliensis* strain 18 in the presence (30 minutes preincubation in PBS) or absence of 100 μg of laminin, with previous treatment with 100 μg of different MAbs or rabbit anti-gp43 serum at 1:100 dilution in PBS. The animals were sacrificed after 2 weeks of infection, testicles were removed, fixed in 10% formalin in BPS, embedded in paraffin, cut in 4-mm sections, and stained with hematoxylin-eosin. Morphometric quantification of the granuloma area in the different experimental groups was carried out using the Mini-mopp system (Kontron Bildanalyse, Image Analysis Systems, Germany) coupled to a standard Zeiss Microscope (Germany), and the results analyzed as described before.¹³

RESULTS

Production of MAbs

After cloning and selection, 12 stable MAbs clones were obtained that specifically recognized the gp43 of *P. brasiliensis*: 40.d7, 17c, 10d, 8a, 21a, 7c, 24a, 27a, 21f, 19g, 32b, and 3e, all characterized as IgG2b with κ light chains.

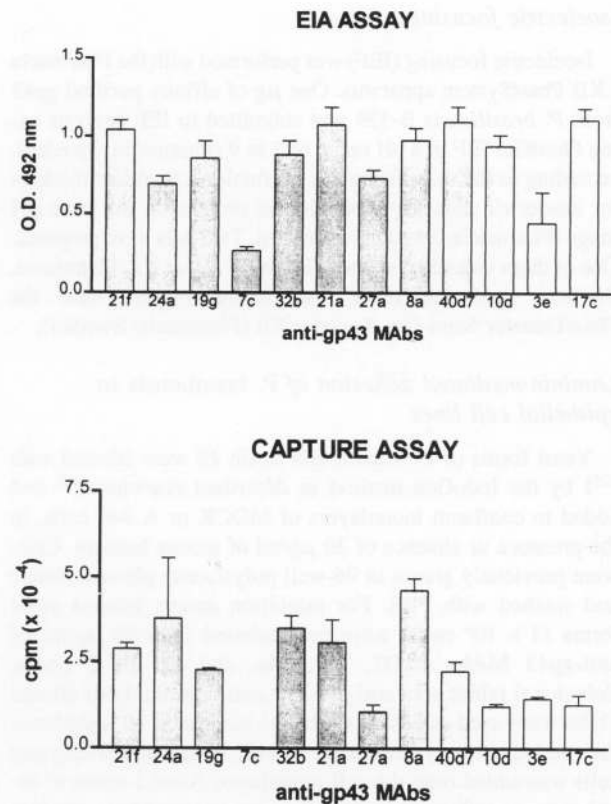


FIG. 1. EIA and Capture assays of MABs showing different levels of reactivity with the gp43. Results represent averages of three determinations. Negative controls (BSA and unrelated MAb 6.C4) and positive control (rabbit polyclonal anti-gp43 serum) are not shown.

EIA, immunoprecipitation, (IP) and capture assays

All MABs recognized gp43 by EIA, immunoprecipitation, and capture assays. Figure 1 shows the results observed by the EIA and capture assays. Best IP results were obtained with MABs 3e and 32b (not shown). Some MABs, such as 19g and 7c, which reacted well in other assays, performed poorly by IP (not shown). To define which MABs were best for use as capture antibodies for serologic purposes, binding assays with labeled gp43 were performed using the MABs in the solid phase. As shown in Figure 1, the best results were obtained with 8a, 32b, and 24a. However, MAB 7c, was shown to be unable to capture antigenic molecules from the liquid phase. No binding was observed with the unrelated 6.C4 MAB or BSA (not shown).

Inhibition assays

No inhibition higher than 20% of the maximal reactivity of MABs in control systems was observed with any of the carbohydrates tested (data not shown). For epitope characterization, inhibition of binding of peroxidase-labeled MAB 17c to gp43 was carried out. Two groups of MABs could be discriminated, one group of MABs very similar to 17c in their affinity for the epitope (MABs 40.d7, 10d, 19g, 27a, 7c, and 3e; data not shown). The other group of MABs (21, 21f, 24a, and 8a) clearly recognized a different epitope in the molecule. MAB 32b reacted in an intermediate fashion. Similar experiments were made by in-

hibition of binding of MAB 21f to gp43, confirming these results (not shown).

SDS-PAGE, IEF, and immunoblottings

All MABs specifically recognized gp43 in immunoblots of the whole exoantigen of *P. brasiliensis*. A representative reaction of anti-gp43 MAB with gp43 is shown (Fig. 2). Also, MABs recognized the deglycosylated form of affinity-purified gp43 (see Fig. 2). Fragments of affinity-purified gp43, partially digested by papain, were recognized by polyclonal anti-gp43 antiserum. Distinct fragments reacted with MABs 40.d7 and 21f (see Fig. 2). Purified gp43 from *P. brasiliensis* strain B-339 showed at least four isoforms, with pIs 6.0, 6.2, 6.6, and 7.

Immunoblotting also showed the specific recognition of all gp43 isoforms by MAB 40.d7 (see Fig. 2) as well as with other MABs such as 19g and 21f (data not shown).

Adhesion of *P. brasiliensis* to epithelial cell lines and inhibition by MABs

Laminin-mediated adhesion of yeast forms of *P. brasiliensis* strain 18 was much more intense onto A549 lung epithelial carcinoma cells than to MDCK cells (Fig. 3). This binding to the A549 cell monolayer was strongly inhibited by MAB 40.d7, and partially by MABs 24a and 17c. In contrast MAB 8a slightly enhanced the adhesion of fungal cells to the human lung cells (see Fig. 3). Similar results, although with lower binding, were observed with MDCK cells (not shown).

Immunofluorescence studies of epithelial cell lines

Both cell lines were shown to be recognized by rabbit anti-laminin anti-sera and both cell monolayers also reacted with laminin (data not shown).

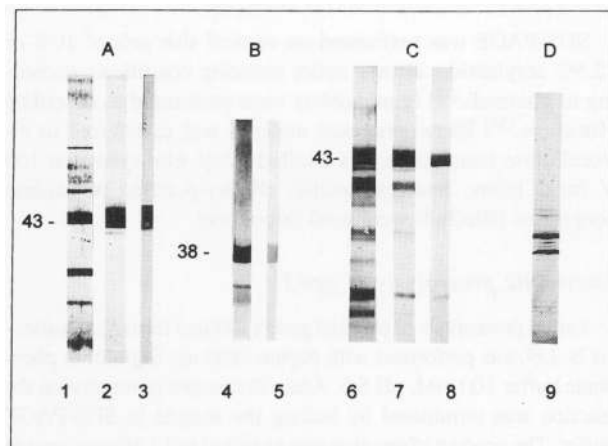


FIG. 2. Reactivity of the gp43 and isoforms, its deglycosylated form and proteolytic fragments with different MABs. SDS-PAGE of whole exoantigen of *P. brasiliensis* (lane 1) and of immunoaffinity purified gp43 with Sepharose-MAB 17c (lane 2); recognition of gp43 by immunoblotting with anti-gp43 MAB (21f) (lane 3); deglycosylated gp43 (lane 4) recognized by anti-gp43 MAB (17c); fragments of papain digested gp43 (lane 5), and their recognition by MABs 40.d7 (lane 7) and 21f (lane 8). Immunoblotting of a set of gp43 isoforms as recognized by MAB 40.d7 (lane 9).

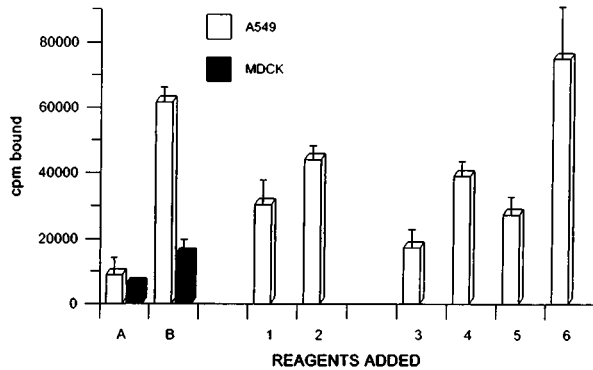


FIG. 3. Binding of ¹²⁵I-labeled yeast forms of *P. brasiliensis* (strain 18) to A 549 (q) or MDCK (n) cells in confluent monolayers. Reactions were performed in the absence (A) or presence of laminin (B; 1 to 7). Inhibition assays were carried out with rabbit polyclonal sera anti-laminin (1) and anti-gp43 (2), or with anti-gp43 MABs 40.d7 (3), 24a (4), 17c (5) and 8a (6).

Influence of anti-gp43 MABs on laminin binding to gp43

Some of the MABs (24A, 17c) were shown to reduce the binding of gp43 to laminin (Fig. 4) whereas MAB 8a was shown to enhance this binding slightly.

Influence of anti-gp43 MABs on laminin-mediated fungal pathogenesis

As previously shown,⁽¹²⁾ laminin coating of yeast forms of *P. brasiliensis* greatly enhanced their pathogenicity in the hamster testicle infection model as compared with the untreated fungus (Figs. 5a and b, respectively). This enhanced pathogenicity was inhibited by the addition of MAB 24a (Fig. 5c) as well as rabbit anti-gp43 anti-serum (not shown). Mab 8a, on the other hand, which had produced a slight increase of adhesion in vitro, showed a massive destruction of the testicle architecture when

Influence of anti-gp43 MABs on the binding of gp43 to ¹²⁵I-laminin

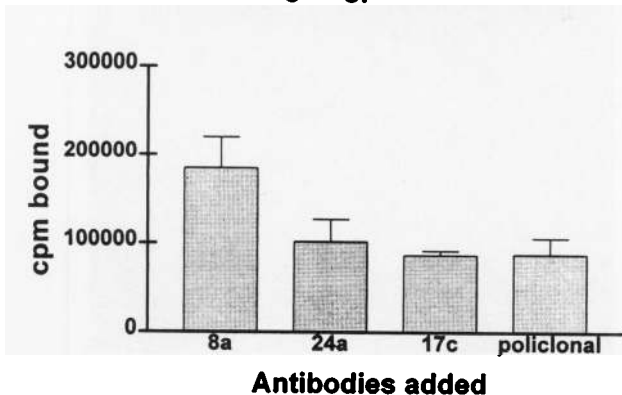


FIG. 4. Influence of anti-gp43 MABs on binding of gp43 to ¹²⁵I-laminin. MABs 24a and 17c, as well as the polyclonal rabbit anti-gp43 anti-serum were inhibitory in contrast with MAB 8a.

added with laminin to the fungal cells before their inoculation in hamsters (Fig. 5d). These results were confirmed by measuring the granuloma area (data not shown). The area occupied by granulomas in the group infected with laminin-coated yeast cells was estimated as $16,34 \pm 7,21 \text{ mm}^2$, significantly larger than that with the untreated fungus ($2,84 \pm 1,56 \text{ mm}^2$). On the other hand, when laminin-coated *P. brasiliensis* strain 18 was incubated in the presence of MAB 24a, the area occupied by granulomas was $2,43 \pm 1,46 \text{ mm}^2$, whereas the group treated with MAB 8a, the granuloma's area was of $13,92 \pm 9,53 \text{ mm}^2$.

DISCUSSION

Paracoccidioidomycosis can be a severe and lethal disease in untreated patients.⁽¹⁾ Its diagnosis is based on fungus isolation or on serologic tests with crude or purified antigens.⁽²⁵⁾ The gp43 is the main purified diagnostic antigen that is specific when used in liquid-phase serologic tests⁽⁴⁾ or when deglycosylated.^(6,7) It is produced by both mycelial and yeast forms⁽²⁶⁾ and seems to be constitutively expressed by the fungus.⁽²⁷⁾ MABs against the gp43 have been used in a capture immunoassay for the specific detection of antibodies from patients with paracoccidioidomycosis.⁽²⁵⁾ Other properties have been attributed to the gp43 in the *P. brasiliensis*/PCM system⁽¹¹⁻¹³⁾ and the MABs presently described have been used to address some of those functional properties of gp43.

In the present work we have characterized 12 MABs against the gp43, all reacting with particular peptide sequences, no significant inhibition being observed with seven different carbohydrates. These results were confirmed by immunoblots with the deglycosylated antigen in which anti-gp43 MABs recognized the 38 kDa component.⁽⁶⁾ All MABs had their isotypes established as IgG2b- κ . These MABs reacted with gp43 by EIA, immunoblotting, and IP, despite some variations in sensitivity depending on the assay used. Inhibition tests with two labeled MABs (17c and 21f) showed that at least three different epitopes can be discerned in the target antigen. MAB 32b was a partial inhibitor possibly because of steric hindrance caused by the proximity of other recognized epitopes. The two main epitopes recognized by MABs on the target antigen were confirmed by testing papain fragments of the gp43, since MAB 40.d7 recognized two fragments of 38 kDa and 28 kDa, whereas MAB 21f recognized only a fragment of 20 kDa fragment. Both MABs were able to recognize the nondigested gp43. These findings are in agreement with the results obtained by Cisalpino et al.⁽²⁸⁾ who analyzed the entire coding region of gp43 and suggested that at least two different B cell epitopes must be present in the gp43 molecule. When tested as capture antibodies for binding the soluble free antigen, 3 MABs (8a, 32b, and 24a) out of 12 showed an affinity higher than the others. MAB 17c has already been used as capture antibody in order to increase the specificity of a serologic test.⁽²⁵⁾ These MABs may also be tested for detection of circulating antigen, which could be of great importance as a criterion of cure in treated patients. An important finding was the demonstration that different isoforms of gp43 from different strains, with p/s ranging from 5.8 to 8.5, can be recognized by MABs 40.d7, 19g, and 21f, suggesting the presence of conserved epitopes in all gp43 isoforms.⁽⁸⁾

Paracoccidioidomycosis is an infection that most probably

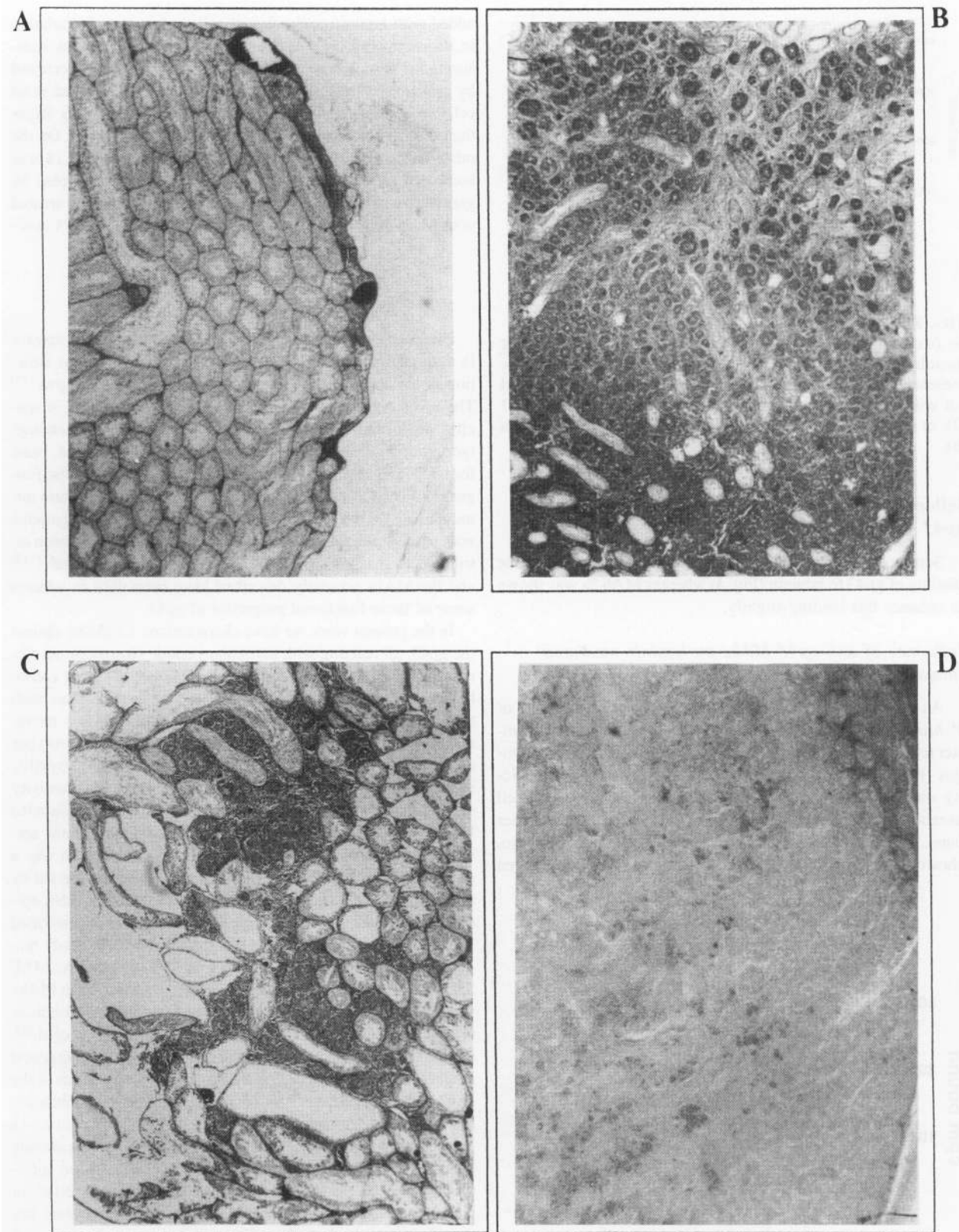


FIG. 5. Histology of hamster testicles after inoculation of *P. brasiliensis*. 10^6 Viable yeast forms of *P. brasiliensis* were injected in the absence or presence of $100 \mu\text{g}$ of mouse laminin (A and B respectively). When treated with MAbs 24a (C) and 8a (D) laminin was added. Animals were sacrificed after 2 weeks of infection, testicles were removed, fixed, cut, and stained by HE ($\times 100$).

begins with the inhalation of fungal propagules, the lungs thus being the portal of entry of the etiologic agents. Fixation of the fungus could depend on its higher affinity for lung epithelial cells. To check this possibility, a comparison of laminin-mediated fungal adhesion to A549 (a human lung carcinoma cell line) and MDCK cells was made. As expected, binding of yeast cells to the lung cells was at least fourfold higher than to canine kidney cells. Although this finding does not demonstrate primary infection of the lungs, it suggests that establishment of the fungus is favored by its binding to the lung's epithelial cells. In those cells both laminin and laminin binding-proteins could be detected by immunofluorescence assay with A549 (not shown). MABs were able to modulate fungal adhesion to the mammalian cells, thus confirming the participation of gp43 as a mediator in this event.

The interaction of fungal cells with the extracellular matrix proteins may contribute for the ability of the infective cells to invade the tissue and disseminate to other organs. This has already been suggested for other infectious agents,⁽²⁹⁻³²⁾ as well as for other fungi such as *C. albicans*,⁽³³⁾ *Aspergillus fumigatus*,⁽³⁴⁾ *Pneumocystis carinii*,⁽³⁵⁾ and *Histoplasma capsulatum*.⁽³⁶⁾ To further address this question, in vivo experiments were performed using the hamster testicle model. We have recently shown that the addition of laminin to the fungal yeast forms before their inoculation greatly enhanced the extension and the severity of the disease,⁽¹³⁾ a result that is presently confirmed. When MAb 24a was added to the fungal suspension, however, the pathogenicity was no longer affected by laminin binding because the granuloma area was comparable to that seen when the untreated fungus was inoculated. Similar results were observed with the rabbit anti-gp43 polyclonal antiserum. The measurement of granuloma-occupied areas in the tissue instead of colony-forming units was used to assess the intensity of infection, this method being reliable and faster.⁽¹³⁾ In contrast, the addition of MAb 8a, which slightly increased fungal adhesion to epithelial cells in vitro, led to a much increased inflammation in vivo, with intense necrotic areas, thus hindering the quantification of the granuloma area. It is conceivable that binding of this MAB to gp43 in situ may somehow change its conformation so as to increase its binding affinity to laminin, with detectable pathogenetic consequences. This has also been suggested by the slight enhancement of ¹²⁵I-laminin binding to gp43 when MAb 8a is introduced in the system.

Taken together, the present results further implicate the gp43 in the *P. brasiliensis* ability to invade the tissues and disseminate to other organs and suggest that blocking its binding to laminin can result in a less severe infection. Gp43 could then represent a possible target for immunotherapeutic studies, and the MABs herein described could be useful for detection of the epitopes involved in the interaction of this antigen with cells and components of the biologic system being studied.

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