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REACTIVITY OF ANTI-GP43 ANTIBODIES FROM *Paracoccidioides brasiliensis* ANTISERUM WITH EXTRACTS FROM CUTANEOUS LESIONS OF LOBO'S DISEASE. PRELIMINARY NOTE

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SUMMARY

We demonstrated through several immunochemical tests the presence of gp43 from *P. brasiliensis* in extracts of cutaneous lesions from Jorge Lobo's disease. This glicoprotein is one of the immunodominant antigens in this species, and is used to identify it.

The demonstration of gp43 tissues infected by the agent of Jorge Lobo's disease is an additional evidence for classifying it in the genera *Paracoccidioides*, species *loboi*.

KEYWORDS: *Paracoccidioides brasiliensis*; Anti-gp43 antibodies; Lobo's disease.

The 43 kDa glycoprotein is the major antigen of *Paracoccidioides brasiliensis* (Splendore, 1912) Almeida, 1930. Its detection guarantees the identification of this species using different immunochemical tests[5]. It has also been demonstrated that antibodies to this glycoprotein are present in the sera of patients with paracoccidioidomycosis, histoplasmosis and Lobo's disease and can be determined by ELISA[6]. Using anti-gp43 and anti-*P. brasiliensis* antisera, SANDOVAL et al.[7] reported that *P. brasiliensis* antigens are present in biopsies from lesions of paracoccidioidomycosis and Lobo's disease. Other data from the literature have demonstrated that the cell walls of the agents of paracoccidioidomycosis and Lobo's disease may express similar constituents (common antigens), the only difference being that *P. loboi* has not yet been cultivated. Recently the gp43 gene from *P. brasiliensis* has been cloned and sequenced by CISALPINO et al.[2].

The present note demonstrates the recognition of a 43 kDa antigen in the extracts of cutaneous lesions from a patient with Lobo's disease, by anti-gp43 antibodies from *P. brasiliensis* antiserum. This reactivity was demonstrated by the following immunochemical tests: a) SDS PAGE[3] on linear 12.5% gel. The antigen was precipitated by trichloroacetic acid (TCA), the precipitate diluted in the sample buffer and 50 µL of this solution applied in each slot of the gel. The staining method used was silver nitrate[1]. b) Immunoblotting[10] carried out with *P. brasiliensis* anti-gp43 polyclonal antiserum produced in rabbit at 1:50 dilution. c) Immunoelectrophoresis[9] with the same reference antiserum. These tests were carried out with the antigen obtained by trypsin treatment (2%) and grinding of biopsies from lesions of patients with Lobo's disease (JL3 somatic antigen). The protein yield, measured by Lowry's method[4] was 1.64 mg/mL. The carbohydrate concentration, measured by Scott & Melvin's method[8] was 0.58 mg/mL. This experiment was made in duplicate.

As control, a cutaneous biopsy from a healthy donor was obtained and similarly processed. All procedures indicated above for detection of gp43 were repeated with this specimen and gave negative results.

Figures 1, 2 and 3 demonstrate the presence of gp43 in the biopsies from patients with Lobo's disease. By immunoelectrophoresis, the cathodal migration[11] arch has a different shape from that obtained with the *P. brasiliensis* antigen, but the precipitation reaction with specific antiserum is clear.

The demonstration of gp43 in human tissue infected by the agent of Lobo's disease represents an additional argument in favor of assigning it to the genus *Paracoccidioides* species *loboi*.

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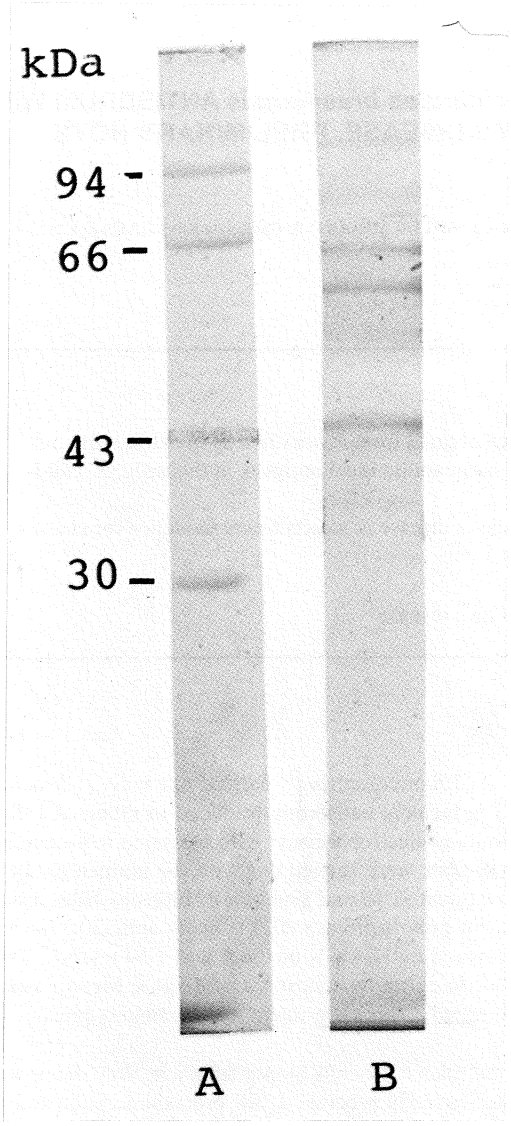

Fig. 1 – SDS-PAGE of biopsy extract (Silver nitrate stain). A) Molecular weight standards; B) Somatic antigen JL3 from *P. loboi*.

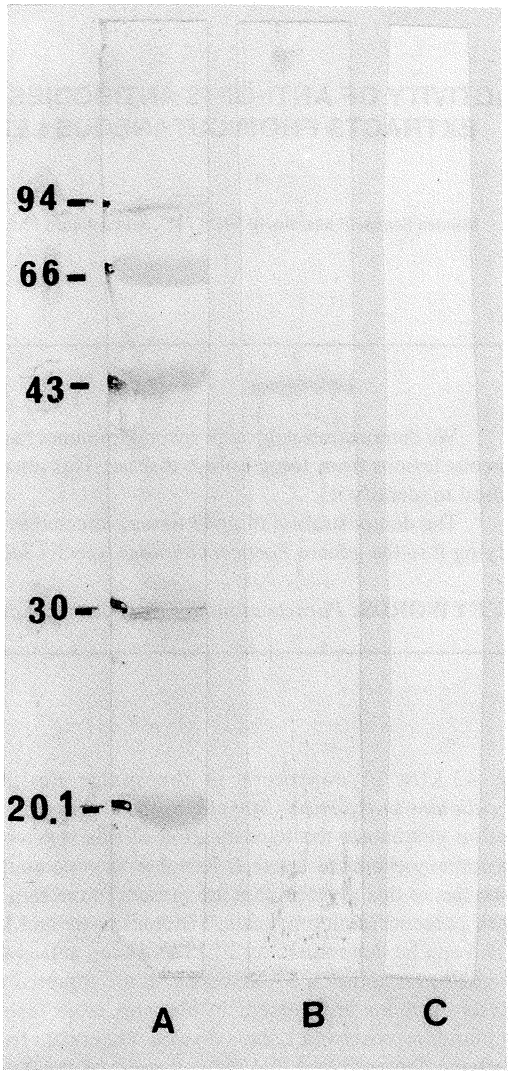

Fig. 2 – Immunoblotting of biopsy extract with anti-gp43 from *P. brasiliensis* antiserum. A) Molecular weight standards; B) Somatic antigen JL3 from *P. loboi*; C) Extract from biopsy of normal skin.

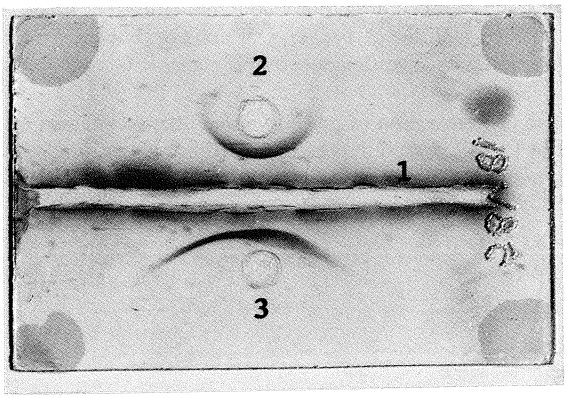

Fig. 3 – Immunoelectrophoresis of antigenic preparation containing gp43 epitopes. 1) Rabbit anti-gp43 antiserum; 2) Somatic antigen JL3 from *P. loboi*; 3) *P. brasiliensis* strain 113 antigen.

RESUMO

Reatividade do soro anti-gp43 do *P. brasiliensis* com extratos de lesões cutâneas da doença de Jorge Lobo

Através de várias provas imunoquímicas foi demonstrada a presença da gp43 em extratos de lesões cutâneas da doença de Jorge Lobo. A glicoproteína de 43 kDa é um dos antígenos dominantes do *Paracoccidioides brasiliensis*, permitindo a identificação desta espécie fúngica.

A demonstração da gp43 em tecidos infectados com o agente da doença de Jorge Lobo, constitui mais um argumento para colocá-lo no gênero *Paracoccidioides*, espécie *loboi*.

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