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Atypical Serological Response Marked by a Lack of Detectable Anti-gp43 Antibodies in a Patient with Disseminated Paracoccidioidomycosis

Mônica S. M. Vidal,[1]*† Gil Benard,[2]† Thales de Brito,[3] Kátia C. Dantas,[1] Cristiane N. Pereira,[1] Francisco O. S. França,[4] Ana M. G. da Silva,[3] and José E. C. Martins[1]

Laboratories of Medical Mycology,[1] Dermatology and Immunodeficiencies,[2] and Immunopathology,[3] Institute of Tropical Medicine, Medical School of the University of São Paulo, Brazil, and Division of Infectious and Parasitic Diseases, Clinics Hospital, Medical School of the University of São Paulo, Brazil[4]

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Serological tests are frequently used to diagnosis paracoccidioidomycosis. A glycoprotein of 43 kDa is considered to be the main diagnostic antigen, being recognized by virtually all patients' sera. A case of atypical serological response, consisting of a lack of detectable anti-gp43 antibodies, in a patient with disseminated paracoccidioidomycosis is presented.

CASE REPORT

The patient, a 34-year-old rural worker from Rondonia state, in the Brazilian Amazon, was admitted to our service on November 2001, with a 6-month history of fever, emaciation, and generalized, fistulous lymphadenopathy (cervical, axilar, and inguinal). Diagnosis was established by visualization of *Paracoccidioides brasiliensis* in secretions and in a lymph node biopsy and by a positive culture from the latter. Chest X rays revealed mediastinal lymphadenopathy and a mild bilateral micronodular interstitial infiltrate. Despite the severity of the disease, results of routine serological tests, double immunodiffusion (DID), and counterimmunoelectrophoresis (CIE) were negative. He was treated with 6 g/day of sulfadiazine with a good response. Five months later, when the clinical manifestations had subsided and the fluctuating lymph nodes had healed, the patient was discharged from the hospital and returned to his original state with a prescription of maintenance treatment with a slow-acting sulfa. However, after having stopped the medication himself, he was readmitted on October 2002 with relapse of the disseminated lymphadenopathy fistulization and pulmonary infiltrate. He was again put on sulfadiazine therapy and exhibited an excellent clinical response. Serological exams (DID and CIE) done in four occasions during this period were negative.

On March 2004, he once more sought our service, at this time presenting with, besides the generalized fistulous lymphadenopathy, dyspnea and ulcerative mulberry-like oral lesions. He had again stopped medication 1 year before. He was again treated with sulfadiazine, with healing of all lesions and improvement of the dyspnea. He completed the attack phase of treatment but was not allowed to return to Rondonia state, being monitored at short intervals at our outpatient unit while on maintenance treatment, presenting as a sequela a mild to moderate pulmonary fibrosis and a mild microstomia.

Diagnosis of paracoccidioidomycosis (PCM) can be made through the visualization of the fungus on patients' samples such as smears of debris from cutaneous lesions, secretions, biopsies, etc. However, serological diagnosis is a frequently used tool for diagnosis, since in many cases the lesions are cryptic. Usually at least two methods, DID and CIE, are recommended to making the diagnosis (13). Specific titration of antibodies is also useful for following up patients under treatment and for determining prognosis of PCM as it correlates with severity of the disease (3, 4).

Studies of the humoral immune response revealed that most of the patients' serum antibodies are directed against a *P. brasiliensis* species-specific component that was first described by Yarzabal et al. (16) as a cathodic arch on immunoelectrophoresis (band E) and was further characterized by Puccia et al. (12) as a glycoprotein of 43 kDa (gp43). It has been claimed that 100% of the PCM patients have anti-gp43 antibodies (15, 11). Four isoforms of gp43 have been described (9). Probing of different isolates with polyclonal animal sera has revealed strain variability regarding the isoforms, which may show differing patterns of seroreactivity (14).

Rarely, a patient may have negative DID and CIE results. However, when further tested by immunoblotting (IB), these patients demonstrate anti-gp43 antibodies (6), probably due to relatively low sensitivity of the gel precipitation tests. In the present case the patient's serum did not recognize the gp43 but revealed anti-70 kDa antibodies (Fig. 1A). This fraction is recognized by 96% of the sera from PCM patients and induces lymphoproliferation, although it is believed not to be species specific (1, 4). It was recently suggested that gp70 facilitates fungal establishment and progression of the disease in an experimental model of primary infection (8).

There are reports of DID false negative results in PCM patients with active infection (5, 10). Do Valle et al. (7) reported that the negative DID tests were more frequent in the juvenile compared with the adult chronic form. In contrast to

* Corresponding author. Mailing address: Instituto de Medicina Tropical de São Paulo, Av. Dr. Enéas Carvalho de Aguiar, 500, andar térreo, CEP:05403-000, São Paulo, SP, Brazil. Phone: 55 11 30667046. Fax: 55 11 30817190. E-mail: movida@usp.br.
† M.S.M.V. and G.B. contributed equally to this work.

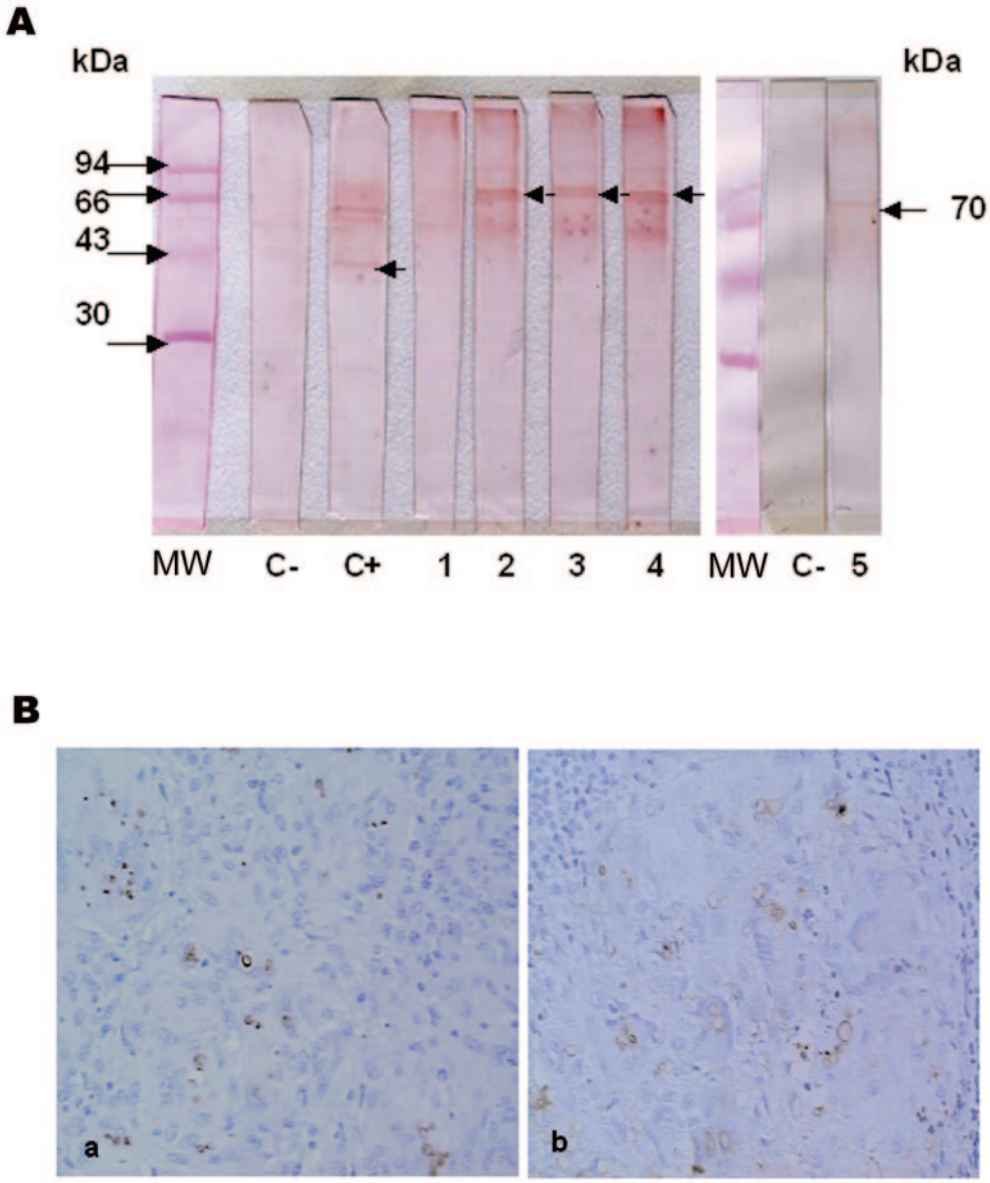

FIG. 1. (A) immunoblotting of serial patient's serum samples with a crude *P. brasiliensis* extract. Lanes: MW, molecular mass standard; C-, blood bank control serum sample; C+, rabbit anti-*P. brasiliensis* hyperimmune serum; 1 to 5, patient's serum samples from, respectively, 11 September 2001, 24 October 2002, 11 May 2002, 12 December 2002, and 11 March 2004. (B) Immunohistochemistry of a lymph node biopsy taken at the first admission, stained with (a) rabbit anti-crude *P. brasiliensis* extract or (b) rabbit anti-gp43 antisera.

their findings, we rarely observe negative DID in the juvenile form, and the few negative DID results are almost exclusively related to patients with the localized, more benign, chronic form of the disease, who are indeed known to produce low levels of antibodies (authors' unpublished data). However, in this setting, immunoblotting usually reveals these low levels of antibodies. Do Valle et al. raised some hypotheses to explain their findings, such as a prozone effect due to excess of antigen secondary to the extensive dissemination of the disease, which we believe to be unlikely in the present case, because this phenomenon is rarely observed in gel immunoprecipitation techniques and because the CIE test is performed employing twofold dilutions of the sera, from 1:1 to 1:256. Formation of immune complexes would be another possibility, but this is not the case in our patient because the negative results persisted on the serial samples obtained later on treatment. Furthermore, the more sensitive immunoblotting will usually resolve the instances where specific antibodies are complexed. In addition, it has also been shown that PCM cases with negative DID present low-avidity immunoglobulin G2 anti-*P. brasiliensis* antibodies that are not revealed by the technique (10). Another difference from our case is that their observations only applied to patients with unifocal disease. Moreover, all patients in their study tested positive when another, more sensitive technique was employed (enzyme-linked immunosorbent assay or IB).

The reasons for the lack of anti-gp43 reactivity in this patient are not clear. It has previously suggested that not all antigenic

epitopes expressed by gp43 are equally present in all *P. brasiliensis* strains (14). This was checked and ruled out by immunohistochemistry studies of the patient's lesion biopsies. We used rabbit anti-gp43 or anti-crude *P. brasiliensis* extract antisera with Envison (K-4011; DAKO Laboratory, Carpenteria, CA) according to manufacturer's instructions, which included the detection of antibody-antigen binding, using 3,3′ diaminobenzidine tetrahydrochloride (D-5637; Sigma, St. Louis, MO) as a chromogen. Staining with the anti-gp43 or anti-crude *P. brasiliensis* extract antisera revealed the same recognition pattern. The testing identified fungal antigens on the cell walls and, in the preserved yeast cells, also in the cytoplasm. The presence of gp43 was observed by immunohistochemistry both in the cytoplasm and in the yeast wall but with predominance in the former (Fig. 1B). Furthermore, the gene for gp43 was identified in the DNA of the patient isolate by a nested PCR performed according to Bialek et al. (2) (data not shown).

On the other hand, the lack of anti-gp43 antibody production could not be ascribed to an immune defect because the patient had no evidence of immunodeficiency. He was human immunodeficiency virus seronegative and had normal numbers of circulating T $CD4^+$, $CD8^+$, and B cells. Besides the anti-gp70 antibody production, he had normal antibody reactivity against common pathogens in our country. Four months after he had begun the current antifungal treatment, with significant clinical improvement, the lymphoproliferative responses to gp43 and gp70 fractions were both undetectable. This is not unexpected, since the patients need prolonged treatment to recover their T-cell responsiveness (1). Nevertheless, the response to the Pokeweed mitogen was in the normal range (data not shown).

This case raises the possibility that, contrary to the current view that sera of PCM patients give positive immunodiffusion or IB tests even when *P. brasiliensis* exoantigen preparations poor in gp43 are used, certain patients may have persistently negative serological results in tests employing this antigen, despite the severity of the disease and the presence of gp43 in their lesions. The present case reinforces the notion that other antigens than gp43, such as gp70, may eventually be of importance as antigenic components of the fungus both in the diagnosis and in the immunopathology of the disease. This must be taken in account when clinicians deal with patients with strong clinical suspicion of PCM but negative serological tests.

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