Rev. Inst. Med. trop. São Paulo
29(5): 327-328, *setembro-outubro*, 1987

DETECTION OF PARACOCCIDIOIDOMYCOSIS CIRCULATING ANTIGEN BY THE IMMUNOELECTROOSMOPHORESIS-IMMUNODIFFUSION TECHNIQUE. PRELIMINARY REPORT.

Nilma Maciel GARCIA, Gilda Maria Barbaro DEL NEGRO, Heidi Pinto MARTINS & Carlos da Silva LACAZ

KEY WORDS: Paracoccidiodomycosis — detection of circulating antigen; Immunoelectroosmophoresis diffusion technique.

Search for circulating antigens has been very often carried out in human infectious diseases, but with relative success. In deep mycoses, such as paracoccidioidomycosis, RODRIGUES et al.[6] reported the presence of a probable circulating antigen in patients' sera by the counterimmunoelectrophoresis technique.

Therefore, we tried to assess three techniques for the search of circulating antigens in patients with paracoccidioidomycosis: modified counterimmunoelectrophoresis, Ouchterlony's double immunodiffusion (ID) and immunoelectroosmophoresis-immunodiffusion (IEOP-ID).

We initially tried to detect a circulating antigen in 115 serum samples from proved cases of paracoccidioidomycosis by a modified counterimmunoelectrophoresis test, using agarose gel at a pH 8.2, as follows: in the anodic well were placed approximately 10 μl of a gammaglobulin fraction obtained by ammonium sulfate precipitation (40% saturation)[7] from a pool of sera containing antibodies to **P. brasiliensis**; in the cathodic well the same volume of a positive serum for paracoccidioidomycosis was placed. In all cases, no precipitin bands or lines appeared whatsoever.

A new experiment was then conducted based on a modification of the classic Ouchterlony's double immunodiffusion (ID) test, placing in the central well a sample of the gammaglobulin fraction and the patients' serum sample around it. Results were negative as well.

We later tried to detect circulating antigens using the immunoelectroosmophoresis-immunodiffusion (IEOP-ID) technique previously described by CONTI-DIAZ et al.[3, 4]. According to this test, for the antibody detection, after the usual passage of the electric current, the serum is again placed for diffusion in the cathodic well of the slide. Through such procedure CONTI-DIAZ et al.[3, 4], were able to show precipitin lines in the cathodic zone, specific for paracoccidioidomycosis.

Following the same procedure, we first carried out a routine counterimmunoelectrophoresis placing 10 μl of the sera pool in the anodic well and 10 μl of the patients' serum in the cathodic one. Further, right after the counterimmunoelectrophoresis, we proceeded with an immunodiffusion wherein 10 μl of the gammaglobulin fraction were placed in a new well on the cathodic side of the same slide. After overnight incubation at 25°C, we noted that two out of 144 examined sera produced a precipitin line of cathodic migration (after amido-black staining), representing a probable circulating antigen, known as YARZÁBAL's E antigen[8, 9] (Fig. 1).

Laboratório de Micologia Médica do Instituto de Medicina Tropical de São Paulo e Laboratório de Investigação Médica (LIM-53, HC-FMUSP). Av. Dr. Enéas de Carvalho Aguiar, 470. CEP 05403 São Paulo, SP. Brasil.

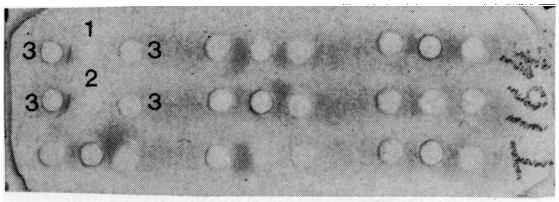

Fig. 1 — Precipitin lines of cathodic migration in serum samples from paracoccidioidomycosis patients. 1 and 2: patients' sera. 3: gammaglobulin fraction obtained from pooled positive sera for **P. brasiliensis.**

It is presumed that larger amounts of circulating antigens are produced in severe forms of the disease in anergic patients, when compared with the hyperergic self-limited infections. Unfortunately, in the present study a correlation between our findings and the patients' clinical data was not possible.

Antigen-antibody circulating complexes are known to occur in cases of paracoccidioidomycosis (RAMOS E SILVA et al.[5], ARANGO et al.[1], CARVALHO et al.[2]).

We plan to carry out the above described procedure in serum samples obtained from experimentally infected animal models, and proceed further in search of the real nature of the precipitin band revealed by the above referred technique.

REFERENCES

1. ARANGO, M.; OROPEZA, F.; ANDERSON, O.; CONTRERAS, C.; BIANCO, N. & YARZÁBAL, L. — Circulating immune complexes and in vitro cell reactivity in paracoccidioidomycosis. **Mycopathologia (Den Haag), 79**: 153-158, 1982.

2. CARVALHO, I. F.; COSTA, J. C.; ROSSI, B. C. & MARTINEZ, R. — Imunocomplexos circulantes em pacientes com paracocciodioidomicose. In: **Encontro sobre paracoccidioidomicose.** 2, Botucatu, 1983. **Anais.** Botucatu, UNESP, 1983. p. 10.

3. CONTI-DIAZ, I. A.; SOMMA-MOREIRA, R. E.; GEZUELE, E.; GIMENEZ, A. C. de; PENA, M. I. & MACKINNON, J. E. — Immunoelectroosmophoresis-immunodiffusion in paracoccidioidomycosis. **Sabouraudia, 11**: 39-41, 1973.

CONTI-DIAZ, I. A.; MACKINNON, J. E.; CALEGARI, L. & CASSERONI, S. — Estudo comparativo de la inmunoelectroforesis (IEF) y de la inmunoelectroosmoforesis-inmunodifusion (IEOF-ID) aplicadas al diagnóstico de la paracoccidioidomicosis. **Mycopathologia (Den Haag), 63**: 161-165, 1978.

5. RAMOS E SILVA, M.; MARQUES, A. S.; CAMPOS, D. S.; TABOADA, D. C.; SOARES, G. H.; BRASCHER, H. M.; VARGENS NETO, J. R.; CRUZ, M. Q. da; LABARTHE, N. V.; ROCHA, G. L. & LIMA, A. O. — Imunologia da paracoccidioidomicose. **An. bras. Derm., 56**: 227-237, 1981.

6. RODRIGUES, M. C.; CASSAGUERRA, C. M. & LACAZ, C. S. — Antigenemia in paracoccidioidomycosis. Probable demonstration of circulating antigen by counterimmunoelectrophoresis test. **Rev. Inst. Med. trop. S. Paulo, 26**: 285-287, 1984.

7. SCOPES, R. — Separation by precipitation. In: CANTOR, R. C. — Protein purification. New York, Springer Verlag, 1982. p. 43-52.

8. YARZÁBAL, L. A. — Anticuerpos precipitantes específicos de la blastomicosis sudamericana revelados por inmunoelectroforesis. **Rev. Inst. Med. trop. S. Paulo, 13**: 320-327, 1971.

9. YARZÁBAL, L. A.; BONT, D.; NAQUIRA, F.; FRINT, J. & ANDRIEU, S. — Identification of the specific antigen of paracoccidioidomycosis responsible for immunoelectrophoretic band E. **Sabouraudia, 15**: 79-85, 1977.

Recebido para publicação em 26/06/87.