

IMMUNOLOGICAL RELATIONSHIP BETWEEN "LEPTOMONAS" *peSSoai* (STRAIN PRINCIPS) *C. fasciculata*, *L. brasiliensis* AND *T. cruzi* BY THE AGAR GEL DIFFUSION TECHNIQUE
PREVIOUS NOTE *

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SUMMARY

The antigens of *C. fasciculata*, *T. cruzi* and *L. brasiliensis* showed cross-reacting precipitin bands with the antigen of *L. pessoai* by the agar gel diffusion technique.

T. cruzi formed two precipitin bands, one of which showed reaction of identity with the antigen of *L. pessoai*.

C. fasciculata produced three precipitin bands, one of which showed immunological identity with the antigen of *L. pessoai*.

L. brasiliensis gave rise to a single band of precipitate which showed identity with the antigen of *L. pessoai*.

INTRODUCTION

In previous papers, a protective effect was demonstrated when mice, treated with *L. pessoai* cultivated "in vitro", were

subsequently infected with virulent strains (strain Y, Berenice) (10). The antigenic community with *L. brasiliensis* was also demonstrated by intradermal tests 1) and indirect immunofluorescence (2).

On the other hand, a number of studies have been developed to disclose the antigenic relationship among different species of trypanosomes and even of different strains of *T. cruzi*. (3,5,7,8 e 9).

Thus it was natural to investigate the immunological relationship of *L. pessoai*, isolated by Galvão et al (6) and trypanosomes pathogenic for man by a technique that permitted to confirm the antigenic identity.

For comparison, we have also tested *C. fasciculata*, a flagellate frequently used in models of laboratory investigations.

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MATERIAL AND METHODS

The following trypanosomatids were studied:

Leptomonas pessoai — Strain principals, isolated from *Zelus leucogrammus* in Goiás in 1968 (6).

Trypanosoma cruzi — Pool of strains Y, Ar, MR, from the Institute of Biological Sciences, Federal University of Minas Gerais. These strains have been maintained in our laboratory in the culture medium of Ducrey.

Leishmania brasiliensis — Pool of strains Acácia, Goiás and Alceu, from the Adolfo Lutz Institute, S. Paulo. It has been cultivated in the medium of Ducrey.

Crythidia fasciculata — Strains from the Institute of Microbiology, Federal University of Rio de Janeiro. It has been kept in our laboratory in a semi-synthetic medium since 1970.

Antigens:

Suspensions of *L. pessoai*, *T. cruzi*, *C. fasciculata* and *L. brasiliensis* have been used in the concentration of $4,4 \times 10^6$ /ml.

L. pessoai and *C. fasciculata* were incubated in a semi-synthetic medium for 48 hours.

T. cruzi was grown in the medium of Barachini for 20 days.

L. brasiliensis was cultivated in the medium of Ducrey for 7 days.

For the preparation of antigens, the strains were centrifuged three times with saline.

The final suspension, containing $4,4 \times 10^6$ /ml, was made in distilled water and 1/10.000

merthiolate was added. The suspension was then frozen at -20.0°C and thawed at room temperature ten times in ten days. It was centrifuged at 10.000 r.p.m. and the supernatant was used as antigen.

Antiserum:

Rabbit anti-*L. pessoai* serum was prepared according to the method described by Coombs and Gell (4) for proteins.

Double gel diffusion:

The Ouchterlony double gel diffusion technique was used to identify the precipitin components produced by the various antigen-antibody systems.

The preparations were kept in a humid chamber at room temperature for 72 hours and then washed and stained according to the Uriel method.

RESULTS

a) — Two bands of precipitate were observed by immunodiffusion of rabbit anti-*L. pessoai* serum and antigen of *T. cruzi*, one of them showing identity and the other presenting nonidentity with the antigen of *L. pessoai*.

b) — By immunodiffusion in agar of *C. fasciculata*, 3 precipitation lines were observed with rabbit anti-*L. pessoai* serum, one of them presenting identity, the two others nonidentity with the *L. pessoai* antigen.

c) — Furthermore, an immunological relationship of *L. brasiliensis* and *L. pessoai* was demonstrated by the same techni-

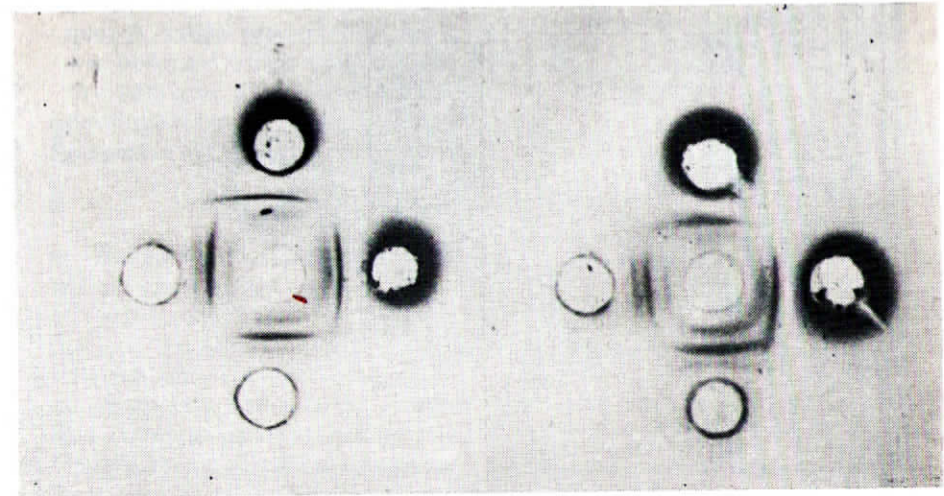


Fig. 1

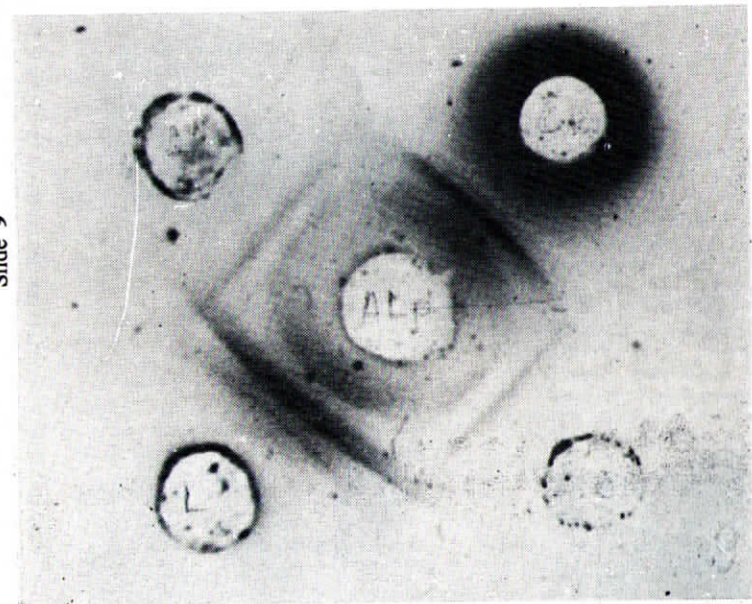


Fig. 2

Fig. 1 — Slide 11 and 12 — Rabbit anti-leptomonas pessoai serum (central well); in the right and top wells, antigen of *C. fasciculata*; in the left and bottom wells, antigen of *L. pessoai* in different concentrations.

Fig. 2 — Slide 9 — Antigen of *L. brasiliensis* (Top left); antigen of *C. fasciculata* (top right); antigen of *L. pessoai* (bottom left); antigen of *T. cruzi* reacting against rabbit anti-leptomonas pessoai serum in the middle well (bottom right).

Slide 3

Slide 7

Slide 4

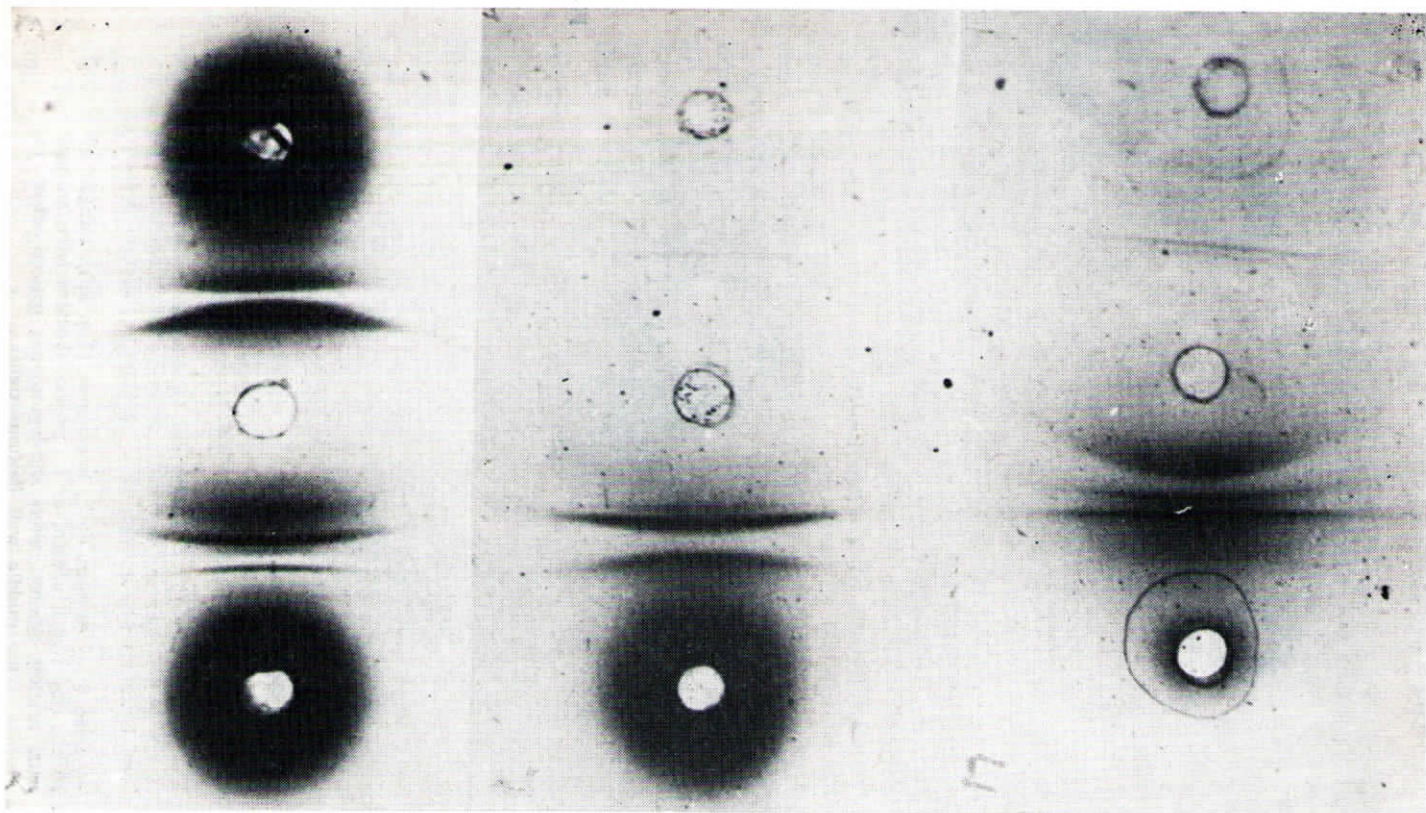


Fig. 3 — Slide 4 — Rabbit anti-leptomonas serum (center); antigen of *L. pessoai* (right); antigen of *T. cruzi* (left).
Slide 7 — Center and right the same as in slide 4; antigen of *L. brasiliensis* on the left.
Slide 3 — In the middle the same as in slide 4; antigen of *L. pessoai* (right); antigen of *C. fasciculata* (left).

que. A single precipitation band was observed which showed identity with one of the three bands developed by the antigen of *L. pessoai*. (Fig. 1, 2, 3)

DISCUSSION

The immunological relationship of different species of the genus *Trypanosoma* has been the subject of intensive studies concerning not only the classification of these species but also the search for common antigens among pathogenic species which may exert a protective effect against pathogenic species.

In a previous work, the partial protective effect of a suspension of *L. pessoai* against a subsequent infection by *T. cruzi* has been demonstrated. These results seem to confirm the experiment "in vitro" inasmuch as the precipitin band of *L. pessoai* antigen presents identity with that of *T. cruzi*.

RESUMO

Usando-se técnicas de imunodifusão em agar-gel, observaram-se faixas de precipitação cruzadas com *L. pessoai* dos seguintes tripanosomídeos: *C. fasciculata*, *T. cruzi* e *L. brasiliensis*. *T. cruzi* apresentou duas faixas de precipitação das quais uma apresentou reação de identidade com antígeno *L. pessoai*. *C. fasciculata* apresentou três faixas de precipitação, uma delas mostrou identidade imunológica com antígeno de *L. pessoai*. *L. brasiliensis* apresentou apenas uma linha de precipitação com identidade com antígeno de *L. pessoai*.

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