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SPECIFIC HUMORAL DEPRESSION IN CHRONIC PATIENTS INFECTED BY TRYPANOSOMA CRUZI

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SUMMARY

We performed a comparative study between xenodiagnosis and serological tests for Chagas'disease. 150 Patients from several endemic areas were studied. Four of them appeared to have a peculiar status with positive xenodiagnosis and negative serology carried out with four classical techniques (Immunofluorescence test, ELISA: Enzyme Linked Immunosorbent Assay, Complement fixation test and Immunoelectrophoresis). One serum out of the four patients presenting humoral depression showed a high quantity of circulating antigen proved by immunoelectrophoresis. The Authors suggest the use of one serological test for detecting circulating antigens of **Trypanosoma cruzi** in addition to the classical serology. It would allow the diagnosis of Chagas' disease in patients with low production of specific antibodies.

INTRODUCTION

Chronic stage of Chagas' disease is characterized by a high production of specific antibodies which allows an easy diagnosis (CAMARGO & TAKEDA[5]). During this stage, several serological tests are available for detecting circulating antibodies, i.e.: complement fixation test, immunofluorescence test, Enzyme Linked Immunosorbent Assay (GUERREIRO & MACHADO[9], LELCHUK et al.[12], VOLLER et al.[21]). Antibodies are present during all the infection and even after treatment (BARCLAY et al.[3], COURA et al.[7]). Xenodiagnosis test allows a parasitological confirmation of the infection, but it is of low sensitivity compared with the serological diagnosis.

We studied 150 chagasic patients proceeding from endemic areas; they were systematically investigated by parasitological and serological tests, looking for a possible correlation between both tests. Among them, four patients presented a peculiar status, with a positive xenodiagnosis and a negative serology.

The Authors discuss the origin of this humoral immunossuppression for total specific antibodies to **Trypanosoma cruzi** in these four cases.

MATERIAL AND METHODS

Patients — 150 patients from endemic areas, having lived for a few years in La Paz City (non endemic area), were investigated by serological diagnosis, and then tested by xenodiagnosis according to the methods described underneath.

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Xenodiagnosis — Patients were exposed for 30 minutes to 30 **Triatoma infestans** specimens of third larval stage. Faeces control was carried out one, two and three months after the insect's bite. This observation was performed on microscope slides pooling faeces from three triatomes.

Serological diagnosis was performed with four techniques:

(1) Immunofluorescence test (IFT) according to WELLER & COONS [22];

(2) Enzyme Linked Immunosorbent Assay (ELISA) according to BOUT et al. [4];

(3) Complement fixation test (CFT) according to GUERREIRO & MACHADO [9] method, modified by KENT & FIFE [10]. A soluble epimastigote antigen was used at a dilution of 0.2 mg/ml;

(4) Immunoelectrophoresis (IEP) was carried out for each serum as described in details by AFCHAIN et al. [1]. The electrophoregrams' interpretation was established with 100 Bolivian sera from non endemic areas and 15 European sera as controls: the test was considered as positive when more than three bands were observed, or only one or two bands if they were strong.

Serological diagnosis was considered as positive when at least three out of the four techniques proved positive, and **vice-versa** for negativity. Criteria for positivity were respectively: titers $\geq$ 1/40 (IFT), optical density $>$ 0.17 (ELISA), titers $\geq$ 1/2 (CF).

Detection of T. cruzi **circulating antigens** — 10 European sera and four sera from chagasic Bolivian patients were tested in IEP against an immune rabbit serum (IRS) obtained by immunization (Vaitukaitis method) with **T. cruzi** antigenic fraction; this fraction was obtained by precipitation of **T. cruzi** total extract with a major immune rabbit serum anti-antigen 5 (LEMESRE [13]).

Isolation and isoenzyme typification of T. cruzi **stocks** — a simple method for obtaining stocks of **T. cruzi** from guts of triatome bug vectors was used (TIBAYRENC et al. [18]). The isoenzyme typification was performed with five enzymatic systems: phosphoglucomutase (E.C.2.7.1., PGM), malate deshydrogenase Nadp+ or malic enzyme (E.C.1.1.1.40., ME), glucose phosphate isomerase (E.C.5.3.1.9., PGI), 6-phosphogluconate deshydrogenase (E.C.1.1.1.44., 6PGD) and isocitrate deshydrogenase (E.C.A.A.A. 42., ICD). The procedures and determination of the zymostrains were according to TIBAYRENC et al. [19].

RESULTS

Results of parasitological and serological examinations for 150 chagasic sera are summarized in Table I. Among the patients presenting a positive serology (97.3%), 61.3% showed a positive xenodiagnosis, and 36% a negative one. These results express the low sensitivity of this parasitological test, in contrast with the serological diagnosis established with four techniques. Four patients out of 96 with positive diagnosis (4.2%) presented a peculiar status, with a positive xenodiagnosis together with a negative serology.

TABLE I

Distribution of patients tested by xenodiagnosis and Chagas'serology

Patients number	Serology	Xenodiagnosis	Percentage of patients
92	P	P	61.3%
54	P	N	36.0%
4	N	P	2.7%

P : Positive
N : Negative

Patient No. 1 had a negative serology, more than one year after a previous positive test with a negative xenodiagnosis which became then positive. Patient No. 2 maintained a negative serology two years after the first test, while the xenodiagnosis, initially positive, had turned negative. Patient No. 3 presented a negative serology one month after a first negative test, and had also a positive xenodiagnosis. Patient No. 4 showed also a negative serology and a positive xenodiagnosis (Table II).

Stocks from patients No. 1 and 3 were typified by isoenzyme technique, confirming their belonging to **T. cruzi** complex (zymostrain 1, TIBAYRENC et al. [19]).

All four sera were investigated looking for circulating antigens of **T. cruzi.** As shown in Fig. 1A and B, serum from patient No. 3 reacts

TABLE II

Serology and xenodiagnosis of four patients with depression of specific humoral immunity to **Trypanosoma cruzi**

	Age	Dates tests	IFT titers	ELISA (D.O.)	IEP (nb. of bands)	Xenodiagnosis
		Nov. 1981	1/40	0.31	2	N
Patient 1	18	Nov. 1982	< 1/40	0.08	0	P
		May 1981	< 1/40	0.02	0	P
Patient 2	48	Jan. 1983	< 1/40	0.09	0	N
		Nov. 1982	< 1/40	0.05	0	P
Patient 3	30	Dic. 1982	< 1/40	0.01	0	—
Patient 4	31	Apr. 1980	< 1/40	0.07	0	P

P : Positive
N : Negative

positively against the IRS, and gives e pattern with 2 precipitation bands. On the other hand, we tested sera from 15 Europeans by the IEP technique; all sera presented with the IRS only one precipitation band, which can be observed also in Patient No. 3. This constitutes probably a non specific reaction between the normal sera and the IRS. The second band, of cathodic localization, was only observed in serum of Patient No. 3, and never in Europeans sera. This band seems specific of **T. cruzi**, and proves the presence of an antigenic component of **T. cruzi** in huge quantity in this patient's serum.

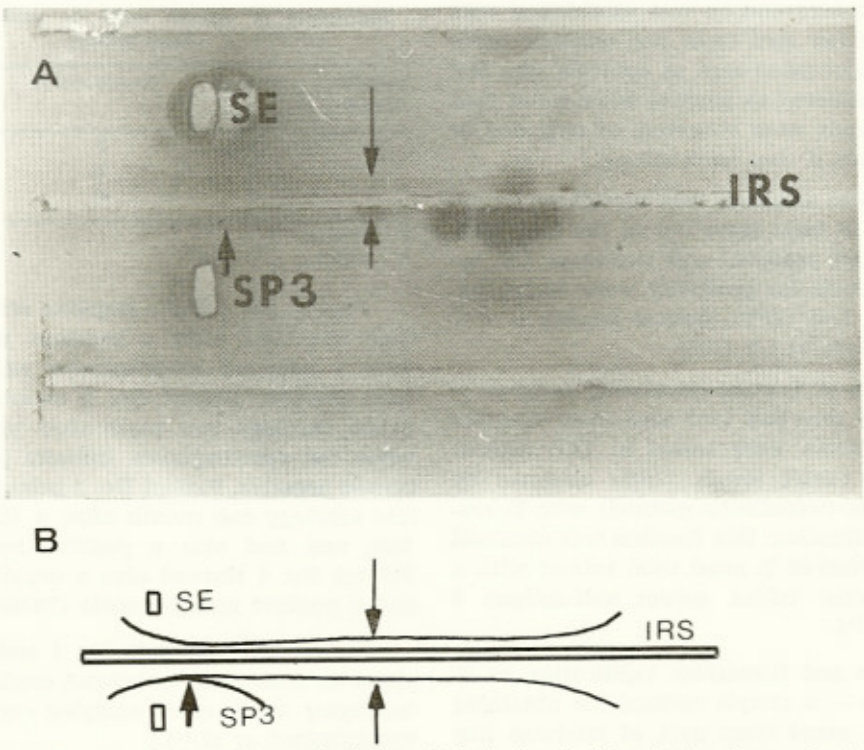

Fig. 1 — **A** and **B** — Picture and diagram of electrophoretic determination of an antigenic component of **T. cruzi** in the serum of patient presenting positive xenodiagnosis and negative serology. Serum of patient No. 3 (SP3), serum of Europeans (SE), Immune Rabbit Serum to an antigenic fraction of **T. cruzi** (IRS), non specific band (⟶), specific band of **T. cruzi** (⟶). All sera were concentrated three times.

DISCUSSION

Our results confirm the supremacy of serology for establishing a diagnosis of Chagas' disease in the chronic stage. In fact, in the studied population, only 61.3% of the patients with a positive serology presented a positive xenodiagnosis. Nevertheless, the four cases we report here were not detected as Chagas' disease by the classical serological tests; on the contrary, in spite of its low sensitivity, xenodiagnosis permitted the diagnosis.

In the present study, a low percentage of patients (2.6%) presented an important depression of specific humoral antibodies' production to **T. cruzi,** but the selection of the patients generally done by the serology does not allow a rigorous evaluation of these cases. Only an epidemiological study, with parasitological and serological diagnosis, could reveal the real frequency of this humoral suppression.

The immunossuppression phenomenons during the evolution of the parasite infections are very frequent and have been demonstrated in various protozoan infections (TERRY [17]). Up to now, non specific immunossuppression in experimental Chagas' disease has been described only during the acute phase of the infection (KIERSZENBAUM [11]). Some Authors assessed that in human infection, a non specific immunossuppression occurs in some acute cases (TEIXEIRA et al. [16]), but probably not in chronic cases (TSCHUDI et al. [20]). However, our results shown in few cases a specific immunossuppression during the chronic period of the infection. This phenomenon could be related to mixed infections (viral, bacterial or parasitological infections associated with Chagas' disease: COX [8], SALAMAN [14], SCHWAB [15]), but in our 4 cases a general clinical examination did not show any intercurrent affection. In addition, the immunossuppression seems to be a lasting phenomenon, one of the patients still presenting a negative serology two years after the first examination. A further immunossuppression study by lymphoblast transformation test would allow to define with more accuracy the origin of this suppression: cellular suppression, or a possible non specific or specific humoral factor such as circulating antigen (CAPRON et al. [6]).

Moreover, the IEP reveals the presence of a specific band to a rabbit immune serum with Patient No. 3 serum; this points to the presence of a rather great quantity of antigen in this serum. The IEP can only detect high quantity of antigenic proteins because of its low sensitivity, which could explain the absence of any band in the sera of patients No. 1, 2 and 4. ARAUJO et al. [2] showed the presence of **T. cruzi** circulating antigens in some sera of chronically infected patients, using ELISA test with Fab'2 coating; this test could be improved using a purified antigen fraction, which could get a more sensitive diagnosis with higher specificity. In these cases with immunossuppression, the systematic investigation of circulating antigens would be useful for Chagas' diagnosis.

RESUMO

Depressão humoral específica em pacientes crônicos infectados pelo Trypanosoma cruzi

Realizamos um estudo comparativo entre o xenodiagnóstico e os testes sorológicos para a doença de Chagas. Cento e cincoenta pacientes de algumas áreas endêmicas foram estudados. Quatro deles pareceram revelar um estado particular com um xenodiagnóstico positivo e uma sorologia negativa, esta realizada com quatro diferentes técnicas clássicas (teste de immunofluorescência, ELISA: Enzyme Linked ImmunoSorbent Assay, teste de fixação do complemento e teste de immuno-eletroforese).

O soro de um dos pacientes que apresentou depressão humoral específica mostra elevada quantidade de antígenos circulantes comprovada pela técnica da immuno-eletroforese. Os Autores sugerem o uso de um teste sorológico para detectar a presença de antígenos circulantes de **T. cruzi,** além da utilização de testes sorológicos clássicos. Isto permitiria o diagnóstico da doença de Chagas em pacientes com uma baixa (ou mesmo inexistente) produção de anticorpos específicos.

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REFERENCES

1 AFCHAIN, D.; CAPRON, A. & PRATA, A. — Les anticorps précipitants dans la Trypanosomiase humaine. **Gaz. Med. Bahia 5:** 141-147, 1970.

2. ARAUJO, F. G.; CHIARI, E. & DIAS, J. C. P. — Demonstration of **Trypanosoma cruzi** antigen in serum from patients with Chagas'disease. **Lancet 8214:** 246-249, 1981.

3. BARCLAY, C. A.; CERISOLA, J. A.; LUGONES, H.; LEDESMA, O.; LOPEZ SILVA, J.; MOUZO, G. & SIERRA, J. P. — Resultados de la actividad anti-**T. cruzi** del Benznidazole en el hombre. **VI Congreso Latino-americano de Farmacologia, Buenos Aires 2:** 9-12, 1976.

4. BOUT, D.; DUGIMONT, J. C.; FARAG, H. & CAPRON, A. — Immunodiagnosis of human parasitic diseases by the Enzyme Linked ImmunoSorbent Assay. **First International Symposium on Immunoenzymatic techniques, INSERM Symposium, Ed. Felman et al. Amsterdam, North-Holland Publishing Company,** 1975, 175-182.

5. CAMARGO, M. E. & TAKEDA, G. K. F. — Diagnóstico de laboratório. **In:** BRENER, Z. & ANDRADE, Z., ed. — **Trypanosoma cruzi** e Doença de Chagas. **1st (ed.). Rio de Janeiro, Guanabara,** 1979, 175-198.

6. CAPRON, A.; CAMUS, D.; DESSAINT, J. P. & BOUBENNEC-FISHER, F. — Altérations de la réponse immune au cours des infections parasitaires. **Ann. d'Immunologie** (Institut Pasteur) **128C:** 541-556, 1977.

7. COURA, J. R.; BRINDERIO, P. J. & FERREIRA, I. — Benznidazole in the treatment of Chagas'disease. Current chemotherapy. **Proceeding of the 10th Intern. Congress of Chemotherapy. Zurich, Switzerland, 18-23 Sept. 1977, 1:** 161-162, **Am. Soc. Microbiol.,** Washington D.C., 1978.

8. COX, F. E. G. — Enhanced **Trypanosoma musculi** infections in mice with concomitant malaria. **Nature** (London) **258:** 148-149, 1975.

9. GUERREIRO, C. & MACHADO, A. — Da reação de Bordet e Gengou na moléstia de Carlos Chagas como elemento de diagnóstico. **Brazil Méd. 27:** 223-226, 1913.

10. KENT, J. F. & FIFE, E .H. — Precise estandardization of reagent for complement fixation. **Am. J. Trop. Med. Hyg. 12:** 103-116, 1963.

11. KIERSZENBAUM, F. — On evasion of **Trypanosoma cruzi** from the host immune response. Lymphoproliferative responses to trypanosomal antigens during acute and chronic experimental Chagas'disease. **Immunology 44:** 641-648, 1981.

12. LELCHUK, R.; DALMASSO, A. P.; INGLESINI, C. L.; ALVAREZ, M. & CERISOLA, J. A. — Immunoglobulin studies in serum of patients with American Trypanosomiasis (Chagas'disease). **Clin. Exp. Immunol. 6:** 548-555, 1970.

13. LEMESRE, J. L. — Specific antigen 5 of **Trypanosoma cruzi:** partial purification and diagnosis application. Molecular and Biochemical Parasitology. **OCIPA V, Toronto 7-14 August, (Suppl.)** p. 668, 1982.

14. SALAMAN, M. H. — Immunodepression by viruses. **Antibiotics & Chemother. 15:** 393-406, 1969.

15. SCHWAB, J. H. — Suppression of the immune response by micro-organisms. **Bact. Rev. 39:** 121-143, 1975.

16. TEIXEIRA, A. R. L.; TEIXEIRA, G.; MACEDO, V. & PRATA, A. — Acquired cell mediated immunodepression in acute Chagas'disease. **J. Clin. Invest. 62:** 1132-1141, 1978.

17. TERRY, R. J. — Immunodepression in parasite infections. **INSERM 72:** 161-178, 1977.

18. TIBAYRENC, M.; ECHALAR, L. & DESJEUX, P. — Une méthode simple pour obtenir directement des isolates de **Trypanosoma cruzi** à partir du tube digestif du triatome vecteur. **Cah. ORSTOM Sér. Ent. méd. et Parasitol. XX:** 187-188, 1982.

19. TIBAYRENC, M.; ECHALAR, L.; LE PONT, F. & DESJEUX, P. — Présence en Bolivie de sept nouveaux variants isoenzymatiques de **Trypanosoma cruzi.** Considérations taxonomiques et émidémiologiques. Discussion sur la valeur antigénique potentielle de certaines isoenzymes. **Cah. ORSTOM, Sér. Ent. méd. et Parasitol.** (sous presse).

20. TSCHUDI, E. I.; ANZIANO, D. F. & DALMASSO, A. P. — Lymphocyte transformation in Chagas'disease. **Infect. Immunity 6:** 905-908, 1972.

21. VOLLER, A.; DRAPER, C.; BIDWELL, D. E. & BARTLETT, A. — Microplate Enzyme Linked ImmunoSorbent Assay for Chagas disease. **Lancet 1:** 426-428, 1975.

22. WELLER, T. H. & COONS, A. H. — Fluorescent antibodies studies with agents of Varicella and Herpes Zoster propagated **in vitro. Proc. Soc. Exp. Biol. (N.Y.) 86:** 789-794, 1954.

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