SIALYLATION PROTECTS TRYPANOSOMA CRUZI TRYPOMASTIGOTES AGAINST LYtic ANTIbODIES IN HUMAN CHAGAS' DISEASE

Pereira-Chioccola, V. L.1,2; Rodrigues, M.M.1; Travassos, L.R.1; Schenkman, S.1

1- Dep. de Microbiologia, Imunologia e Parasitologia - UNIFESP - Escola Paulista de Medicina - R. Botucatu, 862-CEP 04023-062, São Paulo, Brasil.

2-Laboratório de Xenodiagnóstico - Inst. Dante Pazzanese de Cardiologia - Av. Dante Pazzanese, 500-CEP 04012-909

We and others have reported that lytic anti-anti-α galactosyl antibodies (anti-α-Gal) purified from chronic Chagás disease patients recognize anti-α-galactosyl epitopes of O-linked oligosaccharide chains of mucin-like glycoproteins of trypomastigotes. These antibodies strongly agglutinate and destroy trypomastigotes independent of complement. By scanning electron microscopy we found that parasite membrane was dramatically affected by the presence of anti-α-Gal. We have also found previously that most of Chagasic patients present antibodies that
Polymorphism of the Circumsporozoite (CS) protein of *P. vivax* has been described by several authors. Besides the original tandemly repeated amino-acid sequence ("classic" *P. vivax*, Type I), the variant VK247 (Type II) and the one corresponding to human *P. vivax*-like are now known. The latter, morphologically similar to *P. vivax*, has a repetitive sequence identical to that of *P. simiovale*. A previous study from our group in the State of Acre, Brazil, 3056 anophelines captured in 1991-1992 tested by two-site ELISA, found 1.3% positivity for *P. vivax* VK247 and 2.3% for "classic" *P. vivax* in *Anopheles oswaldoi* (n=2610), 0.8% and 0.3% in *An.deaneorum* (n=362), besides positivity for *P.falciparum* (Branquinho et al., 1993). Sporozoites were also found in salivary glands of 1/34 *An.oswaldoi* in the same region, in 1995 (Branquinho et al., 1996). In the present study we evaluated anti-*P. vivax*-like antibodies in human sera from the same region, as well as anopheline infection. Sera were collected from 120 adults of both sexes. Samples were tested by ELISA against the synthetic peptide (APGANQEGGAA). Extracts of 1375 anophelines, which had been kept at -70ºC, from the 91-92 capture, were also processed by capture-ELISA, with monoclonal antibody Pam 172, directed against the same repetitive region of the *P. vivax*-like CS protein. 10.7% (13/120) of the sera tested positive in ELISA. 1207 of these anophelines were *An.oswaldoi* with 12 positives (1.0%), while 168 were *An.deaneorum*, with 2 positives (1.2%). The correlation in the same geographical region between human and anopheline serology for the repetitive CS sequence of human *P. vivax*-like/*P. simiovale* is a strong indication that sporozoites of the variant migrate to the salivary glands of these vectors, and can be inoculated into men. This is an additional indication that *An.oswaldoi* may be a malaria vector in that region and that the parasite is circulating in the area.

Financed by Fundação Nacional de Saúde, LIM49-HC, Superintendência de Controle de Endemias (SUCEN).

---

**SEROLOGICAL DIAGNOSIS OF CUTANEOUS LEISHMANNIASIS BY THE ELISA TECHNIQUE: DEFINITION OF PARAMETERS FOR COMPARING EXPERIMENTAL INFECTIONS IN THE CEBUS APELLA (PRIMATES: CEBIDAE) MODEL WITH NATURAL INFECTIONS IN MAN.**

Souza, R. A¹; Ramos, P.K.¹; Silveira, F.T.¹; Garcez, L.M.¹; Brígido, M.C.²; Muniz, J.P.C.² & Shaw, J.J.¹ & ²

¹ Programa de leishmaniose, Instituto Evandro Chagas/FNS, Av. Almirante Barroso, 492 - 66090-000 Belém, Brazil - email <belproj@amazon.com.br>; ² Centro - Nacional de Primatas/FNS; ³ Depto de Parasitologia, Instituto de Ciências Biomédicas, USP.

One of the axioms for using a non-human primate model to study American Cutaneous Leishmaniasis (ACL) is that their immune responses need to be simialr to those of man. The objective of the present study was to define diagnostic parameters of ACL using the ELISA technique for future comparison with those obtained from experimental infections in *Cebus apella*. The plasmas of 27 patients diagnosed parasitologically as having cutaneous caused *Leishmania (Leishmania) amazonensis* were examined by the ELISA technique. Of these individuals 19 were cases in which the lesions were limited and 8 were cases of anergic diffuse cutaneous leishmaniasis (ADCL). Besides these parasitologically proven cases the serum of 53 normal control individuals with no signs of disease were also tested. ELISA antigens were prepared from cultures of *L. (L.) amazonensis* and *L. (Viannia) braziliensis* by consecutively freezing (-182ºC) and thawing (37ºC) washed cultures 10 times. The
optimal antigen dilutions were determined by testing serial dilutions of known serologically positive sera against
serial antigen dilutions. A human anti-IgG/peroxidase conjugate was used with an OPD substrate and reactions
were read at 492nm. Antibody titres obtained with the different antigens were compared statistically by the
analysis of variance. The ELISA titers of the ADCL patients were significantly higher with the homologous antigen
and were also significantly higher than those of patients with normal ACL. The sensitivity of the test varied in
function of the antigen and was 100% with the \( L. (L.) \) amazonensis antigen and 47.4% with \( L. (V.) \) braziliensis
antigen for patients with normal ACL. The difference between the titers obtained with the two antigens was highly
significant (\( p=0.002684 \)). For normal sera the specificity was 67.9% for the \( L. (L.) \) amazonensis antigen and
86.8% for the \( L. (V.) \) braziliensis antigen. When considering the low specificity for the normal sera, particularly
with the \( L. (L.) \) amazonensis antigen, it has to be remembered that we could not rule out the possibility of
undetected infections. These results show that the ELISA is a sensitive test for uncomplicated ACL caused by \( L.
(L.) \) amazonensis, especially with the homologous antigen but that the specificity of test for human serum needs
to be re-evaluated using normal sera from a non-endemic region.

Financial support: Instituto Evandro Chagas/FNS, Centro Nacional de Primatas/FNS, CNPq., Programa
PCMAM/FNS

COMPARATIVE SEROLOGIC SURVEY OF SERA AND BLOODSPOT ELUATES SAMPLES IN THE DIAGNOSIS
OF CANINE AMERICAN TEGUMENTARY LEISHMANIASIS

Mendes da Cruz, D.A., Duarte, R. & Marzochi, M.C.A.

Laboratório de Imunodiagnóstico, ENSP - FIOCRUZ, Caixa Postal 926, cep. 21041-210, RJ, Brazil

The relation between canine and human leishmaniasis has been shown by the presence of infected dogs in
endemic areas, making evident its importance to the infection epidemiology and explaining the utilization of
canine material in epidemiologic studies. In the serodiagnosis of leishmaniasis, ELISA (Enzyme-linked
immunosorbet assay) and IFT (Indirect immunofluorescent test) have presented satisfactory results, generally
using sera samples in quantitative tests. However, this kind of sample presents difficulties to collection,
transportation and storage. In order to try to solve these problems, blood samples collected on filter paper for
qualitative tests or screenings in epidemiologic studies are currently being used. For elution, filter paper discs
with dried blood samples are cut and put in tubes with phosphate-buffered saline (PBS). In this study we
compared the results of canine sera and bloodspot eluates tested by ELISA-IgG and IFT-IgG for American
tegumentary leishmaniasis (ATL), aiming at a better utilization of this kind of samples on laboratory routine. Both
samples were collected from 72 dogs of São Lourençinho, at Vale do Ribeira, São Paulo State, recognized as
endemic area of ATL and analyzed by IFT-IgG. Only sera samples were analyzed by ELISA-IgG. The results
obtained with sera were 6 (8.3%) positive by IFT-IgG and 11 (15.3%) were positive by ELISA-IgG. The IFT-IgG
performed on bloodspots eluates had 4 (5.5%) positive. The concordance (\( \text{Kappa coefficient} - \kappa \) ) IFT/ELISA
performed on sera was considered regular, and the concordance IFT-sera/IFT-eluates were considered good.
Although IFT-sera is considered the reference method for ATL serodiagnosis, ELISA-sera has shown higher
positivity indices, being indicated for screenings studies, and IFT-eluates in this study showed less sensitivity than
the other methods utilized.