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SERA FROM Trypanosoma b. gambiense INFECTED PATIENTS CROSS-REACT WITH A Trypanosoma cruzi RECOMBINANT PROTEIN.

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Angel Gustavo Guevara E. was a WHO-TDR Scholar and is supported by the Ecuadorian Foundation for Science and Technology (FUNDACYT) to attend the Meeting on Basic Research in Chagas'disease.

In previous studies, we and others have shown the utility of a 24-kDa *Trypanosoma cruzi* recombinant antigen (rTc24) for serological diagnosis of Chagas' disease. Also, this molecule has been proved useful to evaluate cure of chagasic patients who submitt to specific treatment. However, in all the studies done so far, the 24-kDa protein was used as a fusion with a Gluthatione-S-transferase (GST) of *Schistosoma japonicum*, therefore, paralell assays to determine the anti-GST responses of all sera were required to deduce the GST noise in serological tests.

Here, we show the subcloning by polymerase chain reaction of the cDNA encoding the *T. cruzi* 24-kDa antigen in a vector system (pQE) allowing us to obtain Tc24 recombinant protein as a single molecule. The highly reactivity of chagasic sera from Colombia, Ecuador, Brazil and Bolivia in ELISA against the recombinant antigen is confirmed. However, sera from patients infected with African trypanosomes recognize rTc24 in ELISA and blot. The relevance of these findings in the context of Chagas' disease diagnosis and/or the relationship with african trypanosomes is analyzed.

ACKNOWLEDGEMENTS

We thank Dr. A. U. Kretli (FIOCRUZ, Belo Horizonte-Brazil) for brazilian chagasic sera, Dr. R. H. Guderian (Hospital Vozandes, Quito-Ecuador) for ecuadorian chagasic sera, Dr. S. Nicholls (INS, Bogota-Colombia) for colombian chagasic sera and, S. F. Brenière and M. F. Bosseno (CNRS/ORSTOM, Montepellier-France) for bolivian chagasic sera.

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Trypanosoma cruzi RECOMBINANT ANTIGEN AS A TOOL FOR CHAGAS' DISEASE TEST.

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A cDNA library of *T. cruzi* amastigotes, constructed in the vector λgt11, was screened with a pool of sera from Chagasic patients, and one clone (E63) out of 26 highly positives was selected for further characterization. The 2,784 bp insert was subcloned in an expression vector, and the recombinant protein purified to homogeneity. This recombinant protein (pMalE63) was used to induce antibodies, and for evaluation by ELISA technique to detect anti-*T. cruzi* antibodies for the diagnosis of Chagas'disease.

A protein of 80 kda was recognized in Western blot assays by anti-pMalE63 antibodies in amastigotes, trypomastigotes and epimastigotes of *T. cruzi*, and in promastigotes of *Crithidia luciliae*, but there was not recognized any protein in *Leishmania mexicana, L.brasiliensis* and *L. donovani* total extracts. On the other hand, the system ELISA-p MalE63 used for the detection of Chagas' disease, showed a correlation of 96.4% when compared with those serologic technics used as reference, like hemagglutination and immunofluorescence. This correlation was obtained by using 281 sera (58 sera referred as positive, 121 negative chagasic sera to the serologic technics of national reference, and 102 sera from leishmaniasis patients). Statistical analysis of the results showed: sensibility of 93.1%, specificity of 97.3%, predictive positive value of 90% and negative of 98.2%. The kappa index for correlation was 89.2%. which indicates an excellent agreement with the results obtained with those traditional serologic technics.

The above results suggest that the system ELISA-pMalE63 is a good screening tool for its use in blood banks to obtain healthy blood, because the confiability parameters are above 90% when compared with conventional technics used in the diagnosis of Chagas' disease.