

PURIFIED *TRYPANOSOMA CRUZI* SPECIFIC GLYCOPROTEIN FOR DISCRIMINATIVE SEROLOGICAL DIAGNOSIS OF SOUTH AMERICAN TRYPANOSOMIASIS (CHAGAS' DISEASE)

F. GUHL

Laboratorio de Microbiología y Parasitología, Universidad de los Andes, Apartado Aéreo 4976, Bogotá, Colombia

The parasitic protozoan *Trypanosoma cruzi* infects an estimated 10 million individuals in Latin America and a variable proportion of patients can result in a life threatening cardiac or digestive pathology recognized as Chagas' disease. In the majority of patients the parasitaemic phase of infection is transient and often goes unnoticed with the high background of endemic diseases present in the low-income groupings usually infected by *T. cruzi*.

Consequently, diagnosis of infection by direct microscopic examination is rarely possible, and routine serological procedures provide the most sensitive indicator of human infection.

A variety of serological tests capable of detecting early or late infection with *T. cruzi* in man are currently available.

These tests recognized as indispensable epidemiologic and diagnostic tools include complement fixation (CFT) first developed in 1913, indirect immunofluorescence (IFAT), indirect haemagglutination (IHAT) and enzyme-linked immunoadsorbent assay (ELISA) introduced in 1975. It has particular advantages for seroepidemiological surveys and the monitoring of blood donors for Chagas' disease. However, serological tests for Chagas' disease have the disadvantage of cross reactions, especially with *T. rangeli* infections. This parasite have distributions similar to that of *T. cruzi* in most of northern countries of South America like, Colombia, Ecuador, Perú, Venezuela, Panamá and most Central America countries.

Unlike *T. cruzi*, *T. rangeli* is thought to be a harmless parasite of man.

Serological cross-reactions may result in missinterpretation of serological surveys.

Experimental studies have shown that the two trypanosome species share about 60% of their antigenic-make-up (Afchain et al., 1979). Moreover they share the same insect vectors and reservoir hosts. Consequently *T. rangeli* poses a potential problem for the differential immunodiagnosis of *T. cruzi* infection in routine serology.

WHO has long emphasized the need for defined antigens that can be used to distinguish among parasitic infections.

We developed an ELISA system using GP-90 as antigen which seems to fulfil this objective (Schechter et al., 1983).

A lectin-affinity-purified, 90000 molecular weight glycoprotein (GP-90) is present in the known principal strains (zymodemes) of *T. cruzi* and absent from *T. rangeli*.

The GP-90 first described by Snary & Hudson (1979), has been found on blood trypomastigotes, amastigotes and epimastigotes.

The glycoprotein can be purified by lectin affinity chromatography from detergent solubilized epimastigotes. It must have a relatively simple structure when compared to other glycoconjugates present on *T. cruzi*. The glycoprotein contains no phosphate and only 19% by weight of carbohydrate. After purification it can be obtained in milligram quantities from bulk cultures of *T. cruzi* and 2 µg is sufficient for each ELISA.

Initially, serological cross reactions were evaluated in a mouse model in order to be certain that any given individual infected with *T. rangeli* had not been infected with *T. cruzi* and vice-versa (Guhl & Marinkelle, 1982). Sensitivity, specificity and predictive value in this assay were 100%.

Further evaluations including human sera from patients from parasitologically proven cases of *T. cruzi*, *Leishmania donovani*, *L. major*, *L. mexicana amazonensis*, and African trypanosomes, were performed. The sensitivity, specificity and predictive values were, respectively 96.6%, 91.9% and 96.6% (Schechter et al., 1983).

Recent laboratory studies on a selected group of 20 patients from an endemic *T. rangeli*, *T. cruzi* area, showed that 14 gave antibody reactions on immunoassay consistent with *T. cruzi* and/or *T. rangeli*. The use of ELISA GP-90 followed by immuno-precipitation analysis and Western blotting (Guhl et al., 1987), allowed us to diagnose single *T. cruzi* infection, single *T. rangeli* infection and mixed infections.

It seems likely that the true clinical importance of *T. rangeli* infections will only emerge after further study of patients with single and mixed infections.

Antigens purified by affinity chromatography

clearly provide a practical basis for very precise, even strain-specific, diagnostic tests, specially in areas where there is a need to distinguish between pathogenic and non-pathogenic infections.

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