Immunoenzymatic Determination of Antibody-Bound Soluble Antigens of *Trypanosoma cruzi*

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Abstract

Anti-Major Cathodic Antigen (MCA) monospecific immunoglobulins of *T. cruzi* have been used for the preparation of polyamide-linked immunoadsorbents. A high proportion of serologically positive patients with Chagas disease show the presence of a soluble antigen complexed with human immunoglobulins, which complex binds to those immunoadsorbents as determined by a double sandwich reaction and a peroxidase final determination.

Index Entries: *Trypanosoma cruzi,* immunoenzymatic determination of antigens of; Chagas disease, immunoenzymatic determination of antigens of; soluble antigens, of *Trypanosoma cruzi;* immunoenzymatic determination of, *Trypansoma cruzi* antigens.

Introduction

Studies on circulating antigens and immunocomplexes in human trypanosomiasis are important both for the diagnosis of the disease during its chronic phase and for a better understanding of their possible pathogenicity. Some knowledge could also be obtained from this information concerning the cellular injury resulting from the disease (*1–3*). As described by Cossio et al. (*4*), the IVI factor could be a major pathogenic agent of the parasite at the tissue level. This possible action of circulating immunocomplexes significantly increases interest in a specific and sensitive

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method for the detection of *Trypanosoma cruzi* antigens in vivo. The new immunoenzymic tool using isolated recognition sites and specific antigens from the envelope of the parasitic body favors this kind of approach.

Recently Marcipar et al. (*5*) described a procedure for the extraction of glycopeptides from *T. cruzi* epimastigotes and their purification through affinity chromatography on peanut agglutinin (PNA). Highly antigenic glycopeptides, expressed both in culture and in the host, could be obtained. In the present investigation, we attempted to demonstrate that the isolated glycopeptides give rise to circulating immunocomplexes with human immunoglobulins, and that immobilized monospecific antibodies against the same glycopeptides can be used in order to develop an immunoenzymatic method for the detection of these immunoglobulins in human blood sera.

Materials and Methods

"Total glycoprotein extract" (TGE) from *Trypansoma cruzi* Tehuantepec strain was prepared according to BOT et al. (*6*) and Major Cathodic Antigen (MCA) was prepared by affinity chromatography of TGE on Peanut Agglutinin (PNA), as described previously by Marcipar et al. (*5*). Antibodies against the last fraction were obtained in goats, then purified by affinity chromatography on Sepharose, and immobilized on polyamide-6 strips using 1,6-diisocyanohexane as a spacer (*7*, *8*). Rheumatoid factor and other immunoglobulins crossreacting with goat immunoglobulins were eliminated by contact with denaturated goat IgG (*9*). Their absence was checked with latex adsorbed IgG.

Samples of positive sera, checked by the immunofluorescent test, were taken from patients with a chronic phase Chagas disease (provided by Cepialet, Caracas, Venezuela, and CIEN, Santa Fé, Argentina).

Samples of negative sera were taken from European patients (provided by General Hospital, Compiègne, France).

In brief, the determination was performed as follows:

Sera were first deprived of nonspecific crossreacting material by adsorption on denaturated goat IgG. Then, they were diluted from 1/2 to 1/1024. Each dilution was added onto one strip of antibody-bearing polyamide strip of 1 cm^2. Each strip was then washed in PBS + 0.05% Tween 20, and then incubated in a solution of peroxidase-labeled antihuman goat IgG. The strip was washed again and then dipped for 30 min in a solution of OPD peroxidase substrate.

Each sample was assayed in triplicate. The arithmetic mean value of OD was determined using the student *t*-distribution for small samples at a level of confidence of 95%.

Results and Discussion

Table 1 shows the results obtained for the immunoenzymic reaction here described. As it appears from the table, the accepted "positive" and "negative" samples can be differentiated clearly in two populations at the 2.6σ (standard error) level.

TABLE 1
Mean Values and Confidence Interval for Positive and Negative Sera Using Polyamide-Bound Anti-MCA Goat Antibodies as Immunoadsorbents

Sera	$\bar{X}$	Confidence interval, 95%
Positive sera	1.6	1.46–1.737
Negative sera	0.8	0.710–0.890

Compared to the usual response levels of other immunoenzymic tests, the basic level of the negative standard is slightly high. This probably occurs because of immunological side-reactions related to the low specificity of the applied polyclonal monospecific antibody mixture. They should disappear when using a better-isolated specific antibody.

Positive reactions among the healthy population may occur because of a lack of specificity of the reacting antibodies. Negative reactions in Chagas-infected patients may be explained by a lack of or a low level of circulating immunocomplexes. This level may vary during the evolution of the disease.

On the other hand, the cellular reaction against the *Trypanosoma* results in a nearly complete absence of the parasitic body in the blood of the chronically infected patients, so that indirect immunological tests are of major importance for the diagnosis of the disease and for the study of its pathogenesis.

Better specificity can be obtained in the future

(1) By using a better-defined surface antigen as a starting material for the immunization of goats.

(2) By improving the efficiency of the affinity chromatography for the isolation of the goat originated antibody.

A study is going on in order to accomplish these improvements.

Other methods have been used for the detection of immunocomplexes, such as PEG precipitation, labeled C_{1q}, or bovine agglutinin captation. In all cases, largely unspecific results are obtained. In the last two cases, the reaction is dependent on the concentration of available free receptors and on the degree and way of activation of the complement system. A better knowledge of circulating antigens involved in the disease can result from this work, helping in the study of the pathogeny of the disease and of the mechanisms of its spontaneous evolution.

A first screening test on a small population of both patients and healthy people shows that circulating reacting antigens can be detected in most sera of the serogically positive population, and only very few in a negative population. Circulating antigens are recognized by antibodies against the glycoproteic fraction isolated from epimastigote cultures. These antigens are complexed by immunoglobulins G of the serum, which are recognized by antihuman immunoglobulin antibodies. This result is compatible with the idea that circulating immunocomplexes have an antigenic part similar to the glycoproteic extract from epimastigotes (Table 2).

TABLE 2
Correlation between Antibody Response and Investigation of *T. cruzi*-Soluble Immunocomplexes When Using IgG anti-MCA as Immunoadsorbent

No. patients	Antibody IF[a]	Positive reactions	Negative reactions
46	+	38	8
50	−	4	46

[a]Specific-antigen antibodies determined by the immunofluorescence test.

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