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ARTÍCULO ORIGINAL

Protein Profiles of Trypanosoma cruzi and Trypanosoma rangeli

ASTRID J. MEJÍA*, MARÍA TERESA PALÁU*·** y CLAUDIO A. ZÚÑIGA***

ABSTRACT

***Trypanosoma cruzi**, is a protozoan parasite, which has a close phylogenetic relationship with **Trypanosoma rangeli** that is not pathogenic for the vertebrate host. Both parasites have antigenic similarity, they have different and complex total protein profiles according to their morphological and physiological stage epimastigotes or trypomastigotes, showing a differential gene expression during the life cycle. There are also differences according to **T. cruzi** populations used, which were isolated from different geographical areas and were harvested from different sources. This study clearly showed the Colombian SA strain that is highly virulent, has differences in its protein profile when as compared with Dm28c clone, Tulahuen strain and Colombian Astec strain which is not virulent. The proteins were separated by polyacrylamide gel electrophoresis (SDS-PAGE). Specie-specific proteins were found which allow us to identify them, just as it occurs with **T. rangeli** (Choachí-2V strain), which has three protein bands. However, two of them are not only present in epimastigotes but also in trypomastigotes, but the other is exclusive of epimastigote forms.*

Key words: Trypanosoma cruzi*, *Trypanosoma rangeli*, *protein profiles, electrophoresis.

INTRODUCTION

Trypanosoma cruzi (*T. cruzi*) is a protozoan parasite, belongs to the Kinetoplastida order and the Trypanosomatidae family and is the etiological agent of Chagas disease. The parasite has triatomine bugs as insect vectors and develops within insect intestinal tract where undergoes morphological and physological changes.

Trypanosoma rangeli (*T. rangeli*) has a close phylogenetic relationship with *T. cruzi*[1]. This parasite is not pathogenic for vertebrate hosts and presents an overlapping distribution with *T. cruzi*. Both flagellates share several reservoirs and vectors allowing the ocurrence of single and/or mixed infections in both vertebrate and invertebrate hosts throughout the American continent[2]. These mixed infections cause confusion in the serological diagnosis of Chagas disease because both parasites present antigenic

* Departamento de Biología, Facultad de Ciencias, Universidad Nacional de Colombia, Bogotá, Colombia.
** Laboratorio de Parasitología, Instituto Nacional de Salud, Bogotá, Colombia.
*** Unidad de Inmunología, Departamento de Medicina Preventiva, Facultad de Veterinaria, Universidad de Chile, Santiago, Chile.
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Corresponding author: Astrid Johana Mejía M. Carrera 79C Nº 36-73 Sur. Urbanización Francisco José de Caldas, Kennedy. Bogotá, Colombia. E-mail: johanammejia@yahoo.com

cross-reactions that could be demonstrated by numerous laboratory methods[3]. Some authors considered that *T. rangeli* was not able to induce immune response[4],but recent studies have demonstrated that *T. rangeli* induces good immune response[5-6]. It has been postulated that immuno modulating mechanisms induced by *T. rangeli* may have relevance to protection against *T. cruzi* infection[3-6].

Trypanosoma cruzi has the machinery specialized for the host immune system evasion, due to numerous proteins that are in charge of orchestrate the infection success within the vertebrate host. The surface glycoproteins of trypomastigotes are important in the recognition and union of mammalian host cells. In the same way, there are sialidases and trans-sialidases suggested as enzymes involved in the vertebrate host-parasite interaction mechanisms[7-8]. On the other hand, *T. cruzi* expresses at its surface large amounts of mucins, metacyclic mucins are capped and locally shed by the parasite during host cell invasion. Therefore, it is possible that the surface stability during invasion may indicate that specific carbohydrates and binding sites are expressed transiently or that molecular modulation of surface receptors occurs only at certain time points during the invasion cycle by the trypanosomes[9-10].

In the current report we have determinated and compared protein profiles of *T. cruzi* and *T. rangeli* populations harvested from different sources and geographic areas with the purpose of explaining the differences in its total protein profiles and demonstrating specie-specific and stage-specific proteins. In addition, western blot technique was developed to determine the protein recognition using sera from mice infected with *T. cruzi*, *T. rangeli* and mixed infections.

MATERIALS AND METHODS

Parasites stocks - The Tulahuen[11], SA[12] and Astec strains, Dm 28c clone[13] of *T. cruzi* and Choachí-2V strain of *T. rangeli* were used. The Choachí-2V strain was purified by isolation of parasites from haemolymph and salivary glands of *Rhodnius prolixus* experimentally infected with parasites from axenic culture.

Parasites from axenic culture - *T. cruzi* and *T. rangeli* epimastigotes were grown at 25 C in Maekelt and Tobie mediums supplemented with 5% fetal bovine serum (FBS). Flagellates were harvested by pelleting them at 805g for 20 minutes, washing 3 times in cold phosphate-buffered saline (PBS) 0.01 M, pH 7.2, lyophilized and stored at -20 C until used.

Parasite from cell - Metacyclic parasites from axenic culture of each *T. cruzi* population, were poured over a semiconfluent layer of Vero cells in Dulbecco´s Modified Eagle´s Medium (DME) and incubated at 37°C overnight. The medium was then discarded, and fresh DME was added. Eight days after infection, culture medium was collected in a 15 ml centrifuge tube and centrifuged at 805 g for 20 minutes. The supernatant was discarded and the pellet with metacyclic parasites was lyophilized and stored above mentioned.

Vector infection - Thirty fifth instar nymphs of *R. prolixus* maintained in a colony were inoculated by intracelomic route with 2 µl of exponential growth phase axenic culture of *T. rangeli* Choachi-2V strain. Then the triatomines were fed on normal mice and maintained under controlled temperature and humidity conditions. Nine days after infection, metacyclic forms were collected from haemolymph and then lyophilized, stored at cold until used.

Electrophoresis - Lyophilized epimastigote and metacyclic parasites were dissolved in PBS 1X, pH 7.2, then lised by freezing and defrosting for 3 times and lightly vortexed intermittently during this time, and the protein total concentration of all samples was determined by the Bradford method protein assay.

The parasite total extracts of *T. cruzi* and *T. rangeli* were mixed with 4:1 volume of nonreducing Laemmli buffer (0.125 M Tris-HCl pH 6.8, 5% SDS, 20% glycerol, 0.4 bromophenol blue) then were heated at 95 C for 3 minutes. Each protein sample corresponded to a concentration of 200 ug/ml. These suspensions were separated by 8% and 12% sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) as described by Laemmli (1970) using the Mini Protean 3 cell (Bio-Rad)[14]. The samples were electrophoresed under constant voltage (125 V) until the dye front reached the bottom of the gel. Following electrophoresis the gels were stained with Coomassie brillant blue R-280; these gels were then destained, dried and their profiles were analyzed.

Western blot - In addition, we performed

western blotting with total proteins obtained in this study, against sera from mice infected with 4 populations of *T. cruzi* and Choachi-2V strain of *T. rangeli*. Also, we used sera from groups of mice infected with Choachi-2V strain and two weeks later reinfected with each one of 4 populations of *T. cruzi*. These sera were obtained in previous work[12]. Briefly, separated proteins and molecular weight markers were electrophoretically transferred from SDS-PAGE (12%) to 0.45 um nitrocellulose membranes as described elsewhere[15]. Nitrocellulose membrane strips were blocked for 1h with 4% skimmed milk in PBS followed by incubation overnight with sera diluted 1:50 in PBS-Tween. The strips were incubated with appropriated phosphatase-conjugated secondary antibody. The reaction was developed by addition of BCIP and NBT.

RESULTS

Examination of Coomassie-stained SDSPAGE gels revealed that *T. rangeli* and *T. cruzi* display complex protein banding patterns.

T. cruzi from axenic culture - In all *T. cruzi* populations analyzed were found protein bands between 79-80 kDa and 220 kDa. The results showed a protein band of 79-80 kDa for SA strain and Dm 28c clone which migrates as double band with better resolution in 12% gels than 8% gels, similar situation was observed for a 62 kDa protein (Figure 1).

In all the cases, protein bands in the area of 11 kDa to 13 kDa and between 43 kDa and 52 kDa were evident in 12% gels (Figure 1). A 46 kDa protein as double band was found in 8% polyacrylamide-resolving gels for SA and Astec strains and Dm 28c clone, whereas a protein band of 44-45 kDa for Tulahuen strain was evident (Figure 2).

The *T. cruzi* populations studied in the present work showed two protein bands with a molecular weight of approximately 29-30 kDa and 32 kDa in 12% gels, which migrate as double bands in 8% gels (Figure 2).

As shown in Figure 3 the protein bands of 13 kDa, 22 kDa and 35 kDa for SA strain and a band of 24 kDa for Dm 28c clone were found.

T. cruzi from infected cell - The protein profiles of metacyclic parasites showed bands between 36 kDa and 200 kDa. *T. cruzi* populations showed protein bands of 36 kDa, 42 kDa, 110 kDa, 120 kDa and 160 kDa. The protein band of 42 kDa has a similar migration of FBS proteins (Figure. 4). Three protein bands of 54 kDa, 64 kDa and 72 kDa for metacyclic parasites of SA strain were observed in 12% gels (Figure 5).

T. rangeli from axenic culture - We found protein profiles of epimastigotes that showed an evident pattern with 3 bands of 45 kDa, 48 kDa and 53 kDa in 8% and 12 % gels. In addition, other protein bands in the rank of 33 kDa to 200 kDa were observed (Figure 6).

T. rangeli from haemolymph - The protein bands of 48 kDa and 53 kDa were observed whereas a 45 kDa protein was not found in metacyclic parasites of *T. rangeli* isolated from haemolymph. Also we identified tenuous bands in the area of 54 kDa to 200 kDa. These results were similar in protein profiles of epimastigote forms (Figure 6).

Protein bands analysis recognized by sera - Immunoblot analysis revealed that *T. rangeli* proteins with molecular weight of 45 kDa, 48 kDa and 53 kDa, which were observed at electrophoretic profiles, are specie-specific *T. rangeli* antigens. The above mentioned proteins were recognized by sera from mice infected with *T. rangeli* at 15 and 21 days of post-infection and by sera from mice with mixed infection.

When we used homologous serum with *T. cruzi* epimastigotes, the proteins recognized were a set of bands ranging from 80 kDa to 220 kDa and between 26 kDa to 37 kDa; while parasites harvested from infected cell culture revealed mainly high molecular weight bands.

Also, we found antigenic cross-reactivity between *T. cruzi* and *T. rangeli* and were recognized antigens of high molecular weight not only but also antigens of low molecular weight in the area of 25 kDa to 35 kDa mainly (not shown).

DISCUSSION

In the different morpho-physiological stages of *T. cruzi* and *T. rangeli* exist differences in gene expression of stage-specific proteins. It depends on the factors such as parasite necessities and microenvironment conditions.

The 8% gels allowed one better resolution in protein bands separation compared to 12% gels, so it was possible to identify some proteins as

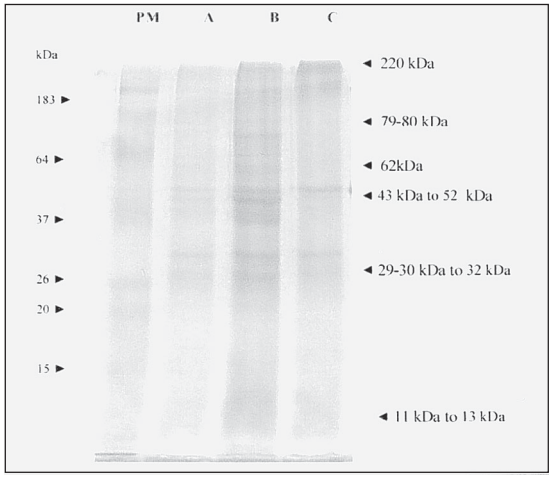

Figura 1. *T. cruzi* from axenic culture A: SA strain; B: Dm28c clone; C: Tulahuen strain. These proteins were run in 12% SDS-PAGE.

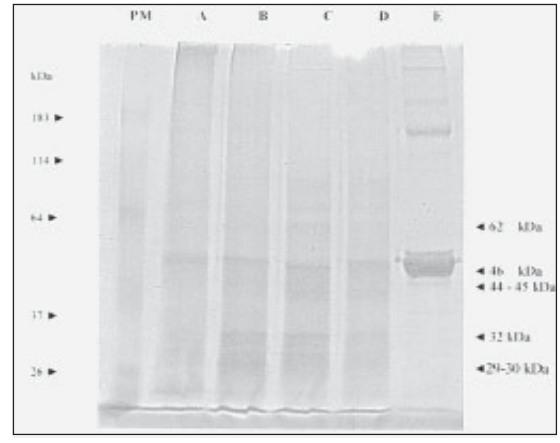

Figura 2. *T. cruzi* from axenic culture A: SA strain; B: Dm28c clone; C: Tulahuen strain; D: Astec strain; E: RE 1 medium. These proteins were run in 8% SDS-PAGE.

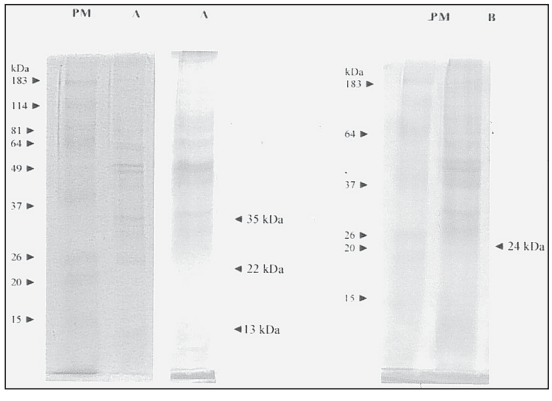

Figura 3. *T. cruzi* from axenic culture A: SA strain; B: Dm28c clone. These proteins were run in 12% SDS-PAGE.

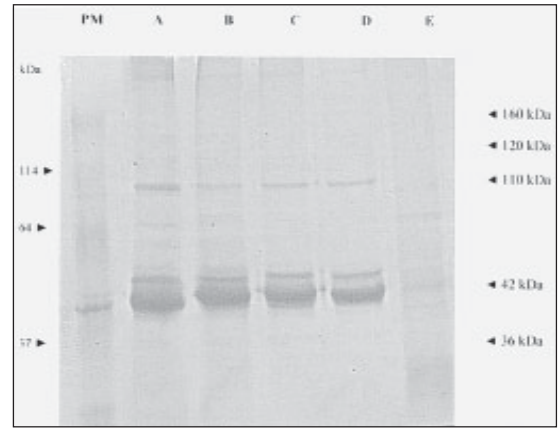

Figura 4. *T. cruzi* from infected cell A: SA strain; B: Dm 28c clone; C: Tulahuen strain; D: Astec strain; E: VERO cells. These proteins were run in 8% gel.

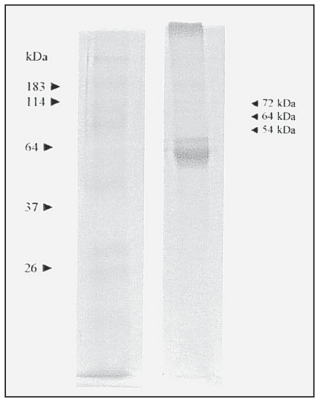

Figura 5. *T. cruzi* SA strain from infected cell. These proteins were run in 12% gel.

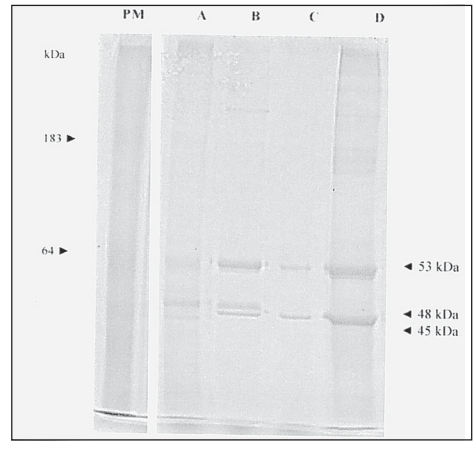

Figura 6. *T. rangeli* Choachí-2V strain: in line A parasites from axenic culture; B: parasites from haemolymph; C: haemolymph from health vector insect; D: *Leishmania sp*. These proteins were run in 8% SDS-PAGE gel. The molecular weights in kilodaltons (kDa) are indicated on the left side and prominent proteins indica-ted on the right side.

doublets. It was the case of 29-30 kDa and 32 kDa in *T. cruzi* populations.

The parasite morphological transformations are associated with specific changes in glycopeptides and polypeptides profiles[16]. The current study shows a complex protein banding profiles in *T. cruzi*, which are different between epimastigotes and trypomastigotes, because we found proteins in the area of 11 kDa to 220 kDa for epimastigote forms and 36 kDa to 200 kDa for trypomastigote forms.

A 160 kDa glycoprotein (gp160) was identified, which is trypomastigote specific. This glycoprotein is attached to the parasite surface membrane by a glycosilphosphatidilinositol (GPI) anchor[17] and is actively released by the parasite into culture media[18].

The results allowed to demonstrate a 79-80 kDa protein, which is near to rank of 83-90 kDa described for proteins implied in recognition and invasion of vertebrate host cells, suggesting these molecules can play an important role in *T. cruzi* structural characteristics[19].

Also, a 120 kDa protein was found, suggesting that this protein may be pteridine reductase that is a enzyme be able to reduce both folates and biopterins, in their reduced forms serve as essential cofactors in a number of critical metabolic steps in *T. cruzi*[20].

The results indicate the presence of mucins, abundant molecules present on the surface of the different stages of *T. cruzi*, with apparent molecular weight of 35kDa-50kDa in epimastigote and 60kDa-200kDa in infected cell culture derived metacyclic trypomastigote[21-9]. The protein bands of 74 kDa, 96 kDa and 120-200 kDa have been reported as mucins[22].

There is strong evidence that the mucins play an important role in the protection against immune system and in host-parasite interaction. These glycoproteins have differences in the degree of glycosylation, therefore different molecular weight. It may also be due to the expression of stage-specific mucin gene products. In fact, mucin genes belong to multigene family[23].

Some proteins of high molecular weight between 79-80 kDa and 220 kDa maybe belong to the trans-sialidase family that are expressed by trypomastigotes and epimastigotes[9] but not find in the intracellular amastigote of *T. cruzi*[24].

The *T. cruzi* and *T. rangeli* proteins have been described antigenically different by several researchers. They are structurally related and their substrates specificities are very similar[25-26]. Also, molecular studies have reported that the sialidases produced by these related parasites exhibit considerable homology when the amino acid sequences are considered[27].

We found a protein with apparent molecular weight of 54 kDa in *T. cruzi* metacyclic forms. This protein is probably cruzipain such it has been reported[28-29].

In our studies, a 24 kDa protein was evident in Dm28c clone; a protein with similar molecular weight was previously described as flagellar protein from *T. cruzi*[18].

Protein bands of molecular weight of 11 kDa and 13 kDa in the *T. cruzi* populations were observed, which are ribosomal proteins as also reported[30].

On the other hand, we found 45 kDa, 48 kDa and 53 kDa proteins for *T. rangeli* with a constant pattern, being 48 kDa protein specie-specific of *T. rangeli*[31].

The protein bands of *T. cruzi* and *T. rangeli* in the rank of 50 kDa to 60 kDa were coincident with the migration of some bands corresponding to FBS as it was informed[32].

Our results confirm that there are several common polypetides between *T. cruzi* and *T. rangeli* and suggested the approximated molecular weight of them. These antigens are able to induce an immune response and that could be involved in the infection and virulence of *T. cruzi*, as well as, they are involved in cross-reactions that causes confusion in the serological diagnosis. With this in mind, the study of these non-specific antigens could be relevant in relation to biochemical/biological comparison of *T. cruzi* and *T. rangeli*.

The difference in the protein profiles of *T. cruzi* and *T. rangeli* that were found in this work suggesting that exist multiple factors such as specie-specific proteins, morpho-physiological stage of the populations and their geographic origin, it indicates complexity in the gene expression of these parasites. Therefore, it is necessary to continue with studies using a wide range of parasite isolates, which allow us to identify and determine the shared proteins by two species. Maybe, these proteins will be involved in the possible protective effect of *T. rangeli* in *T. cruzi* infections.

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