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TRYPANOSOMA CRUZI: EXPRESSION OF ANTIGENIC COMPONENT 5 AMONG 35 LABORATORY CLONES OBTAINED FROM 18 DIFFERENT ISOZYMIC VARIANTS

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SUMMARY

Two monoclonal antibodies anti-component 5 of **Trypanosoma cruzi** (I-35/115 and II-190/30) were tested in IFA and ELISA respectively against 35 **T. cruzi** laboratory clones. Among the 35 clones tested, 18 different isozyme patterns were detected. All clones were recognized by both monoclonal antibodies except one clone which did not react with II-190/30. These results support the universal expression of specific component 5 within the taxon **T. cruzi.**

KEY WORDS: Trypanosoma cruzi: Antigenic components; Clones.

INTRODUCTION

Trypanosoma cruzi, the causative agent of Chagas' disease, infects 24 million people in America [27]. It displays a large heterogeneity in both morphology and biology [8]: for example, differences are observed in virulence, tissue tropism, pathogenicity, drug resistance and antigen composition.

T. cruzi stocks have been characterized taxonomically by means of isoenzyme studies. Analysis of zymograms enabled MILES et al.[13,14] and READY & MILES [16] to distinguish three major groups of isozymic strains which were called "zymodemes". An extensive isozyme study on 121 stocks of **T. cruzi** [25], isolated from various regions of South and Central America, described 43 isozyme variants which exhibited large genetic variability and did not fall into the 3 main clusters that had been proposed initially [13,14,16].

Diagnosis of Chagas' disease during indeterminate and chronic phases is only possible by serologic techniques since circulating levels of parasites are too low. However, techniques used in standard serology are not specific enough to discriminate **Leishmania** and **T. cruzi** infections [2,10]. Furthermore, several mixed infection areas have been found in Central and South America [2,22]. Thus, for diagnostic purposes, for an effective immunological approach to Chagas' disease, it would be important to demonstrate the presence of universal antigens among the taxon **T. cruzi.**

Comparative studies of epimastigote cultures of **T. cruzi** and other **Trypanosomatidae** have demonstrated the existence of a **T. cruzi** specific antigenic component: component 5 [1]. This antigen exhibits several characteristic features. It possesses a great level of immunogenicity in natural[4] or experimental infections as in immunization experiments [1] and has been found at the surface of epimastigotes and bloodstream trypomastigotes [9]. Three murine monoclonal antibodies against component 5 of

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T. cruzi have been purified and characterized [15]. Monoclonal antibody I-35/115 has been shown to bind the epimastigote cell surface (immunofluorescence study) and monoclonal antibodies II-190/30 and II-160/18 to bind internal organelles. Immunoprecipitation of **T. cruzi** iodinated soluble antigen with these monoclonal antibodies, followed by polyacrylamide gel analysis has led to the identification of four molecules whose respective molecular weights are: 72 kD, 51 kD, 43 kD and 24 kD [15]. These proteins were not recognized with the same intensity by the three monoclonal antibodies. Finally, 96.6% of chronic chagasic patient sera have been detected by competitive enzyme immunoassay using anti-component 5 monoclonal antibody (II-190/30). This test is still in evaluation in several laboratories in South America, with W.H.O. grant support.

Here, we evaluate **T. cruzi** component 5 with 2 monoclonal antibodies (I-35/115 and II-190/30) in the taxon **T. cruzi.** Among 35 clones tested, 18 isoenzyme patterns were detected (TIBAYRENC et al. classification, 25), and represent a large proportion of the genotypes classified up to now.

MATERIALS AND METHODS

1 — Parasites:

Parasites were grown in LIT medium. Twenty **T. cruzi** stocks representing 20 different isozymic strains classified according to TIBAYRENC et al. [25] have been cloned by micromanipulation; 35 laboratory clones were so obtained. The original stocks were isolated from mammals or bug vectors from various geographic origins (see Table 1). Control stocks were **Leismania mexicana amazonensis** (WHO IFLA/BR/67/PH-8), **Leishmania brasiliensis braziliensis** (WHO MHOM/BR/75/M-2904), 6 **Leishmania braziliensis braziliensis** Bolivian stocks [7] and **Trypanosoma rangeli** RBG strain.

2 — Isoenzyme analysis of **T. cruzi** clones:

Isoenzyme analysis of the clones was performed using 9 enzyme systems: glucose 6 phosphate dehydrogenase (E.C.1.1.1.49), glucose 6 phosphate isomerase (E.C. 5.1.3.9), glutamate dehydrogenase NADP+ (E.C.1.4.1.4), isocitrate dehydrogenase (E.C.1.1.1.42), malate dehydrogenase (E.C.1.1.1.37), malate dehydrogenase (oxalo-acetate decarboxylating) NADP+ or malic enzyme (E.C.1.1.1.40), phosphoglucomutase (E.C.5.4.2.2, formerly E.C.2.7.5.1), phosphogluconate dehydrogenase (E.C.1.1.1.44) and mannose phosphate isomerase (E.C.5.3.1.8). Methods were described previously [24].

3 — IFA — indirect fluorescent antibody test:

Following the technique described by OROZCO et al. [15], this test was carried out on epimastigote forms in their initial stationary phase. The monoclonal antibody (Mc Ab I-35/115) was used. The conjugate was fluorescein conjugated anti-mice IgG (H+L) (Institut Pasteur Production, Paris, France). All assays were performed in duplicate.

4 — ELISA — Enzyme Linked Immunosorbent Assay:

Epimastigote parasites from the initial stationary phase were washed 3 times in Hanks-Wallace solution, resuspended in NaCl 9% ($100x10^6$ p/100 ul), sonicated and centrifuged at 26,000 g for one hour at 4°C. Subsequent steps have been described previously [3]. Purified Mc Ab II-190/30 was labelled with alkaline phosphatase [12]. All assays were performed in duplicate. Extinction values were measured at 405 nm.

RESULTS

1 — Isozymic variability of **T. cruzi** cloned strains:

Laboratory clones, compared to their original stocks on the basis of isozyme patterns, appeared to be similar in all cases, except one strain with isoenzyme pattern number 19. The original stocks differed for glucose 6 phosphate isomerase (2 bands instead of one observed in 4 clones obtained from this stock). GPI is a dimeric enzyme and the 2 bands obtained for the original stock were probably the result of a mixed population.

2 — Expression of component 5 in clones of **T. cruzi:**

All results are summarized in Table 1. Tested clones were isolated from different mam-

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T A B L E I

Expression of antigenic component 5 in **T. cruzi** isozymic clones

Number of **T. cruzi**	Country of origin	Source of isolates	No. of [a] isozyme strains of original stocks	Mc Ab recognition of Component 5 [b] IFA Mc Ab I-35/115	ELISA Mc Ab II-190/30 Extinction values [c]
1	Chile	Human	43	+	0.43
4	French Guiana	Wild mammal	3	+	1.00
			5 (2 clones)	+	0.22, 0.70
			11	+	0.35
4	French Guiana	**Triatoma** [d]	1 (4 clones)	+	0.73, 0.94, 0.95, 1.02
11	Bolivia	Human	10 (3 clones)	+	0.95, 1.12, 1.60
			16	+	1.47
			19 (5 clones)	+	0.36, 0.43, 0.82, 1.16, 1.48
			39	+	1.01
			39	+	0.72
1	Bolivia	Wild mammal	28	+	0.26
3	Bolivia	**Triatoma infestans**	32	+	0.21
			9	+	0.31
			32	+	0.08
3	Bolivia	**Triatoma** [d]	25 (3 clones)	+	0.75, 1.06, 1.28
7	Brazil	Human	not classified (2 clones)	+	0.26, 1.18
			17	+	1.57
			30 (2 clones)	+	0.9, 1.04
			34	+	1.23
			35	+	1.10
1	Brazil	Wild mammal	36	+	1.25
Control strains					
L. m. a.	Panama		—	—	0.03
L. b. b.	6 Bolivia	4 Human	—	—	0.01, 0.02, 0.03, 0.06
		2 Sandfly	—	—	0.02, 0.07
	1 Brazil	Human	—	—	0.03
T. rangeli	Venezuela	Dog	—	—	0.03

[a] — The isozymic strains were classified according to TIBAYRENC et al.[25]. Among 35 **T. cruzi** clones tested, 18 different isozyme patterns were observed.

[b] — Mc Ab I-35/115 was diluted at 1/10 and 1/25.

[c] — The extinction values of ELISA were from duplicate assays; the limit extinction value was 0.096 determined as m + 3 × (standard deviation) of the 9 control strains.

[d] — **Triatoma** other than **T. infestans**.

mals and vectors. Only one Bolivian clone isolated from **Triatoma infestans** (isozymic strain No. 32) produced a negative result in ELISA with Mc Ab II-190/30. All other clones were recognized by both Mc Ab I-35/115 and Mc Ab II-190/30 tested, respectively, in IFA and ELISA. Extinction values obtained in ELISA, which ranged from 0.08 to 1.57, demonstrate a high level of heterogeneity. IFA controls consisting of a **L. m. amazonensis** strain, 7 **L. b. braziliensis** strains and a **T. rangeli** strain were negative. The limit extinction value of ELISA was determined to be m + 3 times the standard deviation of the 9 control strains: 0.0338 + (3 × 0.027) = 0.096.

DISCUSSION

1 — Clonal diversity in a single isolate of **T. cruzi**:

Our data support the presence of a mixed population in one **T. cruzi** human isolate. This confirms genetic heterogeneity of single **T. cru-**

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zi isolates. Similar findings based on isoenzyme patterns have been reported previously for natural populations of **T. cruzi** isolated from both triatomine bug vectors [23] and humans [5]. All these results emphasize the usefulness of cloning before experimental studies of **T. cruzi** are conducted [1], as recommended by DVORAK et al. [8].

2 — Specific antigens of **T. cruzi** and their diagnostic application:

WHO (1975) [26] recommends the use of specific antigens in the diagnosis of Chagas' disease. Candidate proteins must be expressed in all isoenzyme variants and must be absent in **Leishmania** and **T. rangeli** strains.

Several antigens have been proposed and warrant further research.

a) The 90 kD glycoprotein antigen semi-purified by lectin affinity chromatography was tested in ELISA system [18]. This test is quite sensitive but reacts with sera of patients infected with **Leishmania.** The authors suggest that purification of this antigen is necessary.

b) The 25 kD glycoprotein has been proposed as specific to **T. cruzi**, but was only tested on 8 **T. cruzi** strains [17]. This protein was not detected on either **T. rangeli** or **Leishmania** [17]. 96.5% of chagasic patients sera were positive in immunoprecipitation against this purified protein, and all 23 **Leishmania** human sera were negative in this test [17]. However, no information was given concerning the possible crossed-reactions of these control sera in standard serology for Chagas' disease.

c) A 72 kD surface glycoprotein isolated from both epimastigote and metacyclic trypomastigotes was purified by monoclonal affinity chromatography [20]. Monoclonal antibodies directed against various epitopes of 72 kD glycoprotein have been obtained. These epitopes were shown to be strain- or species specific [6, 11,19]. Some of these monoclonal antibodies are good candidates for use in Chagas' diagnosis.

d) Lastly, our results support the universal expression of **T. cruzi** component 5 among varied set of **T. cruzi** genotypes. Indeed, 18 isozyme variants from different regions and isolate origins were recognized by Mc Ab I-35/115. However, it is worth noting that quantitative differences were observed in the recognition of the clones using the second Mc Ab II-190/30 in ELISA. This could be due to different levels of expression of the epitope recognized by Mc Ab II-190/30. These quantitative differences seem independent of genotype: for genotype 19 we tested 5 clones which range from 0.36 to 1.48, and as great a quantitative difference is observed for genotype 1 (4 clones tested) and genotype 5 (2 clones tested). Both Mc Ab used in this work are directed against a 72 kD glycoprotein (component 5), and the identity of this antigen with the 72 kD glycoprotein of SNARY et al. [20] has been suggested [15]. Nevertheless, further studies are required to show the identity of monoclonal antibodies against the 72 kD protein, and others against component 5. Finally, a specific test using the Mc Ab II-190/30 has been proposed [12]. Our results confirm the utility of this test in specific diagnosis, but appropriate controls of **Leishmania** sera must be tested to ascertain its application in areas with mixed leishmanial and chagasic infections.

RESUMO

Trypanosoma cruzi: **Expressão do componente antigênico 5 entre 35 clones de laboratório obtidos de 18 variantes isoenzímicas.**

Dois anticorpos monoclonais anticomponente 5 de **Trypanosoma cruzi** (I-35/115 e II-190/30) foram testados respectivamente em IFA e ELISA sobre 35 clones de **T. cruzi** isolados no laboratório. Entre estes 35 clones testados, 18 perfís isoenzímicos diferentes puderam ser detectados. Todos os clones foram reconhecidos exceto um clone que não reagiu com o anticor po monoclonal II-190/30. Estes resultados são a favor da expressão constante do componente 5 no seio do taxón **T. cruzi.**

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