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 ex.

T. cruzi have been purified and characterized. Monoclonal antibody 1-35/115 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. 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 (1-35/115 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. 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 Leishmania mexicana amazonensis (WHO IFLA/BR/67/PH-8), Leishmania braziliensis braziliensis (WHO MHOM/BR/75/M-2904), 6 Leishmania braziliensis braziliensis Bolivian stocks and Trypanosoma rangeli RBG strain.

2 — Idenzyme 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), isocitra-

### TABLE I
Expression of antigenic component 5 in T. cruzi isozymic clones

<table>
<thead>
<tr>
<th>Number of T. cruzi</th>
<th>Country of origin</th>
<th>Source of isolates</th>
<th>No. of isozyme strains of original stocks</th>
<th>Mc Ab recognition of Component 5</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>IFA Mc Ab I-35/115 ELISA Mc Ab II-190/30 Extinction values</td>
</tr>
<tr>
<td>1 Chile</td>
<td>Human</td>
<td>43</td>
<td></td>
<td>+ 0.43</td>
</tr>
<tr>
<td>4 French Guiana</td>
<td>Wild mammal</td>
<td>1 (2 clones)</td>
<td></td>
<td>+ 1.00</td>
</tr>
<tr>
<td>11 Bolivia</td>
<td>Human</td>
<td>10 (3 clones)</td>
<td></td>
<td>+ 0.22, 0.70</td>
</tr>
<tr>
<td>1 Bolivia</td>
<td>Wild mammal</td>
<td>25</td>
<td></td>
<td>+ 0.35</td>
</tr>
<tr>
<td>3 Bolivia Triatoma</td>
<td>22</td>
<td></td>
<td></td>
<td>+ 0.21</td>
</tr>
<tr>
<td>7 Brazil Triatoma</td>
<td>25 (3 clones)</td>
<td></td>
<td></td>
<td>+ 0.26</td>
</tr>
<tr>
<td>1 Brazil Wild</td>
<td>26</td>
<td></td>
<td></td>
<td>+ 0.75, 1.06, 1.22</td>
</tr>
</tbody>
</table>

Control strains

- L. m. a. Panama — — 0.03
- L. b. b. 6 Bolivia 4 Human 2 Sandfly 1 Brazil Human — — — — 0.01, 0.02, 0.03, 0.06 0.02, 0.07 0.03
- T. rangeli Venezuela Dog — — 0.03

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

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

WHO (1975) 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. 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. This protein was not detected on either *T. rangeli* or *Leishmania*. 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. 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. Monoclonal antibodies directed against various epitopes of 72 kD glycoprotein have been obtained. These epitopes were shown to be strain- or species-specific. 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 isoenzyme 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 1 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. has been suggested. 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. 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 isoenzimáticas.

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 perfil isoenzimáticos diferentes puderam ser detectados. Todos os clones foram reconhecidos exceto um clone que não reagiu com o anticorpo monoclonal II-190/30. Estes resultados são a favor da expressão constante do componente 5 no seio do taxon *T. cruzi*.

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REFERENCES


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