TABELA I: Porcentagem de diferenciação celular das cepas Be-62 e Be-78 de *Trypanosoma cruzi* cultivadas em células “Vero” a 33°C

<table>
<thead>
<tr>
<th>Cepa</th>
<th>Forma evolutiva</th>
<th>Dias após a infecção</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>4º</td>
</tr>
<tr>
<td>Be-62</td>
<td>Amastigota</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>Tripomastigota</td>
<td>0</td>
</tr>
<tr>
<td>Be-78</td>
<td>Amastigota</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>Tripomastigota</td>
<td>0</td>
</tr>
</tbody>
</table>

Nos experimentos realizados com a cepa Be-78 foi observado que a interação parasita x célula é mais lenta podendo-se verificar taxas mais elevadas de diferenciação no 6º dia de infecção (Tabela 1).

- **Infecção em Camundongos Albinos e C₃H Isogênicos**

  Foi avaliada a infecção de camundongos albinos e C₃H inoculados com tripomastigotases sangüíneas e com tripomastigotases provenientes de cultivo celular. Para esse estudo foram avaliados os seguintes parâmetros: (1) a morfologia das formas sangüíneas; (2) a parasitemia; (3) a taxa de mortalidade; (4) exames anatomopatológicos.

  O estudo da morfologia das formas sangüíneas demonstrou que a cepa Be-62 apresentou predomínio de formas delgadas em todas as situações estudadas à semelhança do descrito pela literatura (Tabela 2).
TABELA 2: Porcentagem de tripomastigotas delgados, intermediários, largos e muito largos observados em sangue de camundongos albinos inoculados com tripomastigotas provenientes de cultivo em células Vero (7º dia) da cepa Be-62 de *Trypanosoma cruzi*.

<table>
<thead>
<tr>
<th>Dia após a inoculação</th>
<th>Morfologia das formas sangüíneas</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Delgadas</td>
</tr>
<tr>
<td>4º</td>
<td>91,3</td>
</tr>
<tr>
<td>5º</td>
<td>88,0</td>
</tr>
<tr>
<td>6º</td>
<td>65,6</td>
</tr>
<tr>
<td>7º</td>
<td>63,0</td>
</tr>
<tr>
<td>8º</td>
<td>57,0</td>
</tr>
<tr>
<td>9º</td>
<td>60,3</td>
</tr>
<tr>
<td>10º</td>
<td>47,3</td>
</tr>
<tr>
<td>11º</td>
<td>43,3</td>
</tr>
</tbody>
</table>

Com a cepa Be-78, só foi possível avaliar a morfologia das formas sangüíneas após a passagem por células “Vero”, quando mudou de comportamento em camundongos, passando a apresentar nestes animais, alta parasitemia e mortalidade, fatos nunca observados anteriormente. Nestas condições foi encontrado um predomínio de tripomastigotas largos e a existência de formas muito largas (Tabela 3).
TABELA 3: Porcentagem de tripanomastigotas delgadas, intermediárias, largos e muito largos observados em sangue de camundongos albinos inoculados com tripanomastigotas provenientes de cultivo em células Vero (8º dia) da cepa Be-78 de *Trypanosoma cruzi*.

<table>
<thead>
<tr>
<th>Dia após a inoculação</th>
<th>% de tripanomastigotas sanguíneos</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Delgadas</td>
</tr>
<tr>
<td>8º</td>
<td>56</td>
</tr>
<tr>
<td>9º</td>
<td>36</td>
</tr>
<tr>
<td>10º</td>
<td>17</td>
</tr>
<tr>
<td>11º</td>
<td>16</td>
</tr>
<tr>
<td>12º</td>
<td>7</td>
</tr>
<tr>
<td>14º</td>
<td>13</td>
</tr>
<tr>
<td>15º</td>
<td>7</td>
</tr>
<tr>
<td>16º</td>
<td>6</td>
</tr>
<tr>
<td>17º</td>
<td>3</td>
</tr>
<tr>
<td>18º</td>
<td>3</td>
</tr>
<tr>
<td>19º</td>
<td>4</td>
</tr>
</tbody>
</table>

Após uma única passagem da cepa Be-78 por células “Vero” (CT-1), foi observada uma alteração no comportamento biológico desta cepa em camundongos (albinos e C3H) inoculados com tripanomastigotas provenientes de cultura celular (TCC). Foi observado um aumento da parasitemia (Gráficos 1 e 2) e da taxa de mortalidade. O tempo de cultivo em células “Vero” levou à ligeira diminuição da virulência da cepa. Camundongos inoculados com tripanomastigotas derivados da CT-6 e CT-12 apresentaram menor parasitemia (Gráficos 1 e 2) e menores taxas de mortalidade do que os animais inoculados com tripanomastigotas da CT-1.

A cepa Be-62 apresentou, após uma única passagem em células “Vero”, mudanças mais discretas nos parâmetros estudados. Também com esta cepa o tempo de cultivo em células “Vero” diminuiu ligeiramente a parasitemia, sem contudo alterar a taxa de
mortalidade que continuou acontecendo em 100% dos animais, embora mais tardiamente (Gráficos 3 e 4).

Ambas as cepas apresentaram aumento de patogenicidade para camundongos albinos e C3H isogênicos, ao longo da fase aguda, após “passarem”, por células “Vero”, especialmente após as primeiras passagens.

A cepa Be-78 apresentou aumento mais acentuado de sua patogenicidade para camundongos, após passagem por células “Vero”. Animais inoculados com esta cepa apresentaram ninhos de amastigotas gigantes, mais freqüentes e de aspecto muito diferente do observado na situação controle de manutenção da cepa através de passagens sanguíneas sucessivas.

**Objetivo II:** Estudar os clones obtidos das cepas Be-62 e Be-78 antes e após cultivo em células “Vero”, comparativamente com as cepas parentais.

Foram realizadas clonagens de ambas as cepas utilizando o parasito cultivado em meio acelular (LIT), os clones obtidos foram expandidos em meio LIT, e o “pellet” estocado a -70°C.

Foram realizadas várias clonagens de tripomastigotas provenientes de células “Vero” e sangue. Em nenhuma das tentativas de clonagem com estas formas foram obtidos clones de T. cruzi. Sendo assim, o trabalho está sendo prosseguido apenas com os clones obtidos através da clonagem do parasito proveniente de meio acelular (LIT).

**Objetivo III:** Determinação do Perfil de Isoenzimas e de DNA

A análise do perfil de Isoenzimas revelou que a cepa Be-62 antes de passar por células “Vero” pertence ao Zimodema A ao se considerar as oito enzimas testadas (PGM, MDH, GPI, ME, G6PD, ASAT, ALAT e PGD). Já a cepa Be-78 nas mesmas condições, apresentou perfil de Zimodemas B ou C nas enzimas PGM e MDH.

A análise do perfil de DNA de ambas as cepas (Be-62 e Be-78) antes de passarem por células “Vero”, revelou diferenças marcantes entre a cepa Be-78 (em diferentes situações de infecção em camundongos) em relação à cepa Be-62 quando se utilizou a enzima ECO-RI.

A análise do perfil de Isoenzimas e do de DNA dos clones obtidos das cepas parentais está sendo realizada.
Gráfico 1: Curvas de parasitemia da cepa Be-78 de Trypanosoma cruzi, realizadas em camundongos albinos inoculados com tripomastigotas sanguíneos (controle) e com tripomastigotas provenientes de células Vero na 1ª, 6ª e 12ª passagem (CT).
Gráfico 2: Curvas de parasitemia da cepa Be-78 de Trypanosoma cruzi, realizadas em camundongos C3H isogênicos inoculados com tripomastigotas sanguíneos (controle) e com tripomastigotas provenientes de células Vero na 1ª, 6ª e 12ª passagem (CT).
Gráfico 3: Curvas de parasitemia da cepa Be-62 de Trypanosoma cruzi, realizadas em camundongos albinos inoculados com tripomastigotas sanguíneas (controle) e com tripomastigotas provenientes de células Vero na 1ª, 6ª e 12ª passagem (CT).
Gráfico 4: Curvas de parasitemia da cepa Be-62 de *Trypanosoma cruzi*, realizadas em camundongos C3H isogênicos inoculados com tripomastigotas sanguíneas (controle) e com tripomastigotas provenientes de células Vero na 1ª e 12ª passagem (CT).
Publicações Resultantes do Projeto

Trabalho Científico

Lana, M.; Chiari, C.A.; Chiari, E.; Morel, C.M.; Gonçalves, A.M.; Romanha, A.J. Characterization of two isolates of Trypanosoma cruzi obtained from the Berenice, the first human case of Chagas’ disease described by Carlos Chagas in 1909. Parasitol Res., 1996.

Apresentação em Congressos


Obs: O trabalho definitivo está sendo elaborado para ser publicado em revista indexada.
BI-036
CHANGE OF BEHAVIOUR OF TWO STRAINS OF TRYPANOSOMA CRUZI AFTER CULTIVATION IN VERO CELLS

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Previous studies have shown that two strains of T. cruzi isolated from the Berenice patient, considered the first human case of Chagas' disease (Chagas, 1909), respectively in 1962 (Be-62) and in 1978 (be-78) are distinct in relation to theirs biological and biochemical (isoenzymes and restriction endonucleases, K-DNA, profiles) parameters.

Comparative studies of these two strains in Swiss and C3H mice showed remarkable differences between them. Be-62 strain presents a very high parasitemia with peak on the 8th day after infection (DAI) and 100% of mortality until the 20th day. However, Be-78 strain showed a low parasitemia with peak around 12th DAI, patent parasitemia until around day 30 and no mortality during the acute phase of the disease.

We present here data showing that these strains behave differently in mice after cultivation in “vero” cells. Intracut of 50,000 trypomastigotes of both Be-68 and Be-78 strains derived from the 1st, 6th and 12th passages in “Vero” cells in Swiss and C3H mice induced an acute phase of the disease more severe than that observed by blood trypomastigotes inoculation.

Be-78 strain showed an increased parasitemia ranging about 50 times greater than blood forms infection after the 1st passage in “Vero” cells and a rate of mortality of 100% never observed before. With Be-62 strain these alterations were not significative. Mice infected with trypomastigotes of both strains, derived from the 6th and 12th passages in “Vero” cells, showed a gradual decrease of parasitemia and mortality.

The histopathological studies with Be-78 strain infections were always compatible with the parasitological findings. A severe pathological damage and parasitism, mainly in the myocardium and skeletal muscle, was also observed. The morphology of the amastigote nests as well as their number and size were always large and different from those observed in mice infected with blood trypomastigotes during successive passages.

These data suggest the occurrence of mixed infection of T. cruzi in the patient Berenice. Both strains are being cloned to verify this hypothesis.

Supported By CNPq, FAPEMIG and UFOP
Characterization of two isolates of *Trypanosoma cruzi* obtained from the patient Berenice, the first human case of Chagas' disease described by Carlos Chagas in 1909

Abstract Two isolates of *Trypanosoma cruzi* were obtained from the patient Berenice, the first human case of Chagas' disease (Chagas 1909), when she was 55 and 71 years old, respectively. The isolates were characterized on the basis of their epimastigote-trypomastigote differentiation in liquid media and of the electrophoretic pattern of Ector1 digestion products of kinetoplast DNA (k-DNA) minicircles (schizodeme) and isoenzyme patterns (zymodeme). Clear differences were found between the isolates, suggesting the occurrence of a heterogeneous population of *T. cruzi* in the infection of this patient.

Introduction

Chagas' disease, an important health problem in Latin America, was discovered in Lassance, Minas Gerais state, Brazil, by Carlos Chagas (1909) when he examined the 2-year-old girl Berenice, describing the first human case of the disease. Afterward, two isolates of *Trypanosoma cruzi* were obtained from her through xenodiagnosis. The first isolate was obtained when she was 55 years old (Salgado et al. 1962) and the second, when she was 71 years old (Lana 1981). On both occasions, Berenice was carefully examined and was considered normal in relation to all clinical forms of Chagas' disease (Salgado et al. 1962). She died at the age of 73 years, apparently due to causes other than Chagas' disease (Rocha 1992). Berenice exhibited a balanced human host-*T. cruzi* relationship and constitutes the best example of the indeterminate form of Chagas' disease (Garnham 1980).

The isolate obtained by Salgado et al. (1962) has been characterized and studied in many aspects by different authors (Brener 1965; Brener et al. 1974; Melo and Brener 1978) and has been further compared with the isolate obtained in 1978 (Lana 1981; Lana and Chiari 1986). In this study the production of trypomastigotes in liquid media and the zymodeme and schizodeme of both isolates were analyzed.

Materials and methods

Isolates of *Trypanosoma cruzi*

Two *T. cruzi* isolates were obtained via xenodiagnosis from the patient Berenice. The Be-62 isolate was obtained by Salgado et al. (1962) and isolate Be-78, by Lana (1981). Feces from infected triatomines used for xenodiagnosis were inoculated and maintained in mice. Furthermore, the isolates were reobtained from infected mice through hemoculture in liver infusion tryptose (LIT) medium. Be-62 was isolated in the 4th passage in albino mice. Be-78 was isolated in the 1st and 20th passages in C3H mice, in the 1st and 40th passages in albino mice, and in the 6th cyclical passage in C3H mice alternated with nymphs of the vector *Dipterogaster maximus*.

Differentiation in liquid media

*T. cruzi* epimastigote-to-trypomastigote differentiation was evaluated when the samples were in the stationary phase of growth, between the 10th and 20th passages in culture. An inoculum of 2.5 x 10^7 parasites/ml was used in LIT (Camargo 1964) and M16 (Chiari and Camargo 1984) media. These experiments were performed on 10-ml cultures in 50-ml Erlenmeyer flasks incubated at 28°C. Differentiation was assessed every 2 days by differential counting of the flagellates in Giemsa smears. The results were expressed as the percentage of trypomastigotes found in 500 randomly counted flagellates. The experiments were carried out in triplicate and repeated at least twice.
Isoenzyme analysis

The cultures were established in LIT media under stationary growth conditions at approximately 2 months after isolaion from infected mice. Approximately 30 ml of culture containing 5.0 x 10^7 flagellates/ml was used. The flagellates were washed with phosphate-buffered saline (PBS) by centrifugation at 4°C and the pellet was stored at -70°C. The enzymatic extracts were obtained at 4°C according to Kilgour and Godfrey (1973). The lysates were centrifuged at 15,000 g for 1 h at 4°C. The supernatant, called the enzymatic extract, was cryopreserved in liquid nitrogen. Isoenzymes were separated by refrigerated horizontal thin-layer starch-gel electrophoresis. The enzymes studied were glucose-6-phosphate dehydrogenase (G6PD, EC.1.1.1.49), alanine aminotransferase (ALAT, EC.2.6.1.2), aspartate aminotransferase (ASAT, EC.2.6.1.1), malic enzyme (ME, EC.1.1.1.40), phosphoglucomutase (PGM, EC.2.7.5.1), glucose phosphate isomerase (GPI, EC.5.3.1.9), malate dehydrogenase (MDH, EC.1.1.1.37), and 6-phosphogluconate dehydrogenase (6PGD, EC.1.1.1.44). As references, T. cruzi strains from zyomodes A, B, C, and D were used (Carneiro et al. 1990).

Schizonted analysis

The pelot of parasites obtained previously was used for extraction and purification of kinetoplast DNA (K-DNA; Gonçalves et al. 1984). K-DNA was digested with EcoR1 for 1.5 h at 37°C. K-DNA fragments were separated by vertical electrophoresis in 6-10% polyacrylamide gradient slab gels. The fluorescence patterns were visualized and photographed after staining with ethidium bromide and transillumination with UV light.

Results

Differentiation in liquid media

Be-62 showed a lower percentage of epimastigote-to-trypanomastigote differentiation than did Be-78 (Fig. 1). Both isolates presented higher differentiation rates in M16 medium than in LIT medium. Nevertheless, Be-62 showed the same differentiation profile in M16 medium as did Be-78 in LIT medium. Similar results were also obtained when parasites were harvested from infected mice at different numbers of blood passages (data not shown).

Isoenzyme patterns

Be-62 showed typical patterns of zymodeme A for all enzymes tested. Be-78 displayed typical patterns of zymodeme A in six of the eight enzymes tested. However, PGM and MDH showed a pattern typical of zymodeme B (Fig. 2). The conditions of maintenance and the number of successive blood passages did not change the isoenzyme profiles.

![Fig. 1](image1.png)  ![Fig. 2](image2.png)
the eight enzymes, the exceptions being PGM and MDH. With these enzymes, Be-78 showed profiles from zymodeme B. Our data suggest the occurrence of a heterogeneous population of T. cruzi in the infection of the patient Berenice. Similar results were observed by Romanha (1982) in some chronic chagasic patients of Bambuí city, Minas Gerais state, Brazil. It cannot be excluded that Berenice may have acquired a second infection with a certain strain of T. cruzi. It also cannot be excluded that during the life of Berenice, some T. cruzi parasites may have been eliminated step by step by her immune system such that the Berenice strains isolated from the same patient at different times show different biochemical characteristics.

Many authors have described the isolation of heterogeneous T. cruzi populations from the same patient as well as other vertebrate and invertebrate hosts. Morel et al. (1980) have demonstrated that the standard CL "strain", isolated from a Triatoma infestans, is actually a mixture of at least two subpopulations that differ in their biological and biochemical properties. Marques de Araujo and Chiari (1988) cloned this strain and encountered biological and biochemical differences among the subpopulations. Brenière et al. (1985, 1989) showed that 13.0% and 12.3%, respectively, of chagasic Bolivian patients were infected with at least two different T. cruzi zymodemes. These results were observed after the parasites had been obtained either through the same tritoma or via different tritomine specimens used for the same xenodiagnosis. There is also experimental evidence of the coexistence of more than one strain of T. cruzi in the same host. Deane et al. (1984) demonstrated this finding in mice inoculated with the Y and F T. cruzi strains through schizodeme and zymodeme analysis. Alves et al. (1993) described reversible changes in the isoenzyme patterns and infectivity to mice of clones obtained from the standard T. cruzi Y strain after its maintenance under different conditions in the laboratory.

It has been shown that zymodeme A is very common in chronic patients in many regions of Brazil (Romanha 1982; Schlemper 1982). This zymodeme is equivalent to the zymodeme Z2 from the domestic cycle (Miles et al. 1981; Luqueti et al. 1986) reported in different regions of Brazil.

The two isolates Be-62 and Be-78 were also different when the k-DNA profile was analyzed using EcoRI. This parameter confirms and extends the characterization by isoenzyme analysis (Carnieiro et al. 1990). Neither the type of maintenance nor the number of successive passages in mice changed the zymodeme or the schizodeme of either isolate.

Taken as a whole, these results address some intriguing questions concerning the T. cruzi-human interaction:

1. Was the patient Berenice infected once or at different times by more than one clone or population of T. cruzi?

2. Did the process of isolation (xenodiagnosis) select different T. cruzi populations on each occasion?
REFERÊNCIAS BIBLIOGRÁFICAS


A cepa Be-78 de T. cruzi tem apresentado ao longo de sua manutenção em camundongos albinos e C3H isogênicos, predomínio de formas largas, alta infectividade, baixa parasitemia e virulência com total sobrevida dos animais à fase aguda da infecção. Em ambas as raças de camundongos foi sempre observado tropismo dos parasitas para o miocárdio e musculatura esquelética, acompanhado de alterações muito discretas. No entanto, em cães, esta cepa tem se mostrado muito virulenta desenvolvendo a cardiopatia fibrosante intensa em todos animais autopsiados durante a fase crônica da infecção.

Resultados preliminares mostram que a cepa Be-78 altera todas as suas características após uma única passagem por células “Vero”. 13C. Grupos de camundongos de ambas as raças, inoculados com 50.000 tripanastigotas derivados destas células (TCT), apresentam grande aumento da parasitemia (aproximadamente 20 vezes) e mortalidade de 60% em camundongos albinos e de 100% em camundongos C3H, durante a fase aguda da infecção. As lesões encontradas nestes animais, são de evolução e intensidade completamente diferentes do observado anteriormente sendo encontradas: 1) intenso parasitismo tecidual (nínios de parasitas muito volumosos, rotos ou não e com parasitas em diferentes estágios de evolução) especialmente no miocárdio; 2) miocardite e/ou miosite (focal e/ou difusa e intensa com predominância de pequenos linfócitos e macrófagos; 3) fenômenos regresivos intensos (lesão de Magatúes Torres, mioticólise e necrose).

No entanto, se tripanastigotas destes camundongos albinos e C3H (inoculados com TCT) são inoculados em grupos de camundongos de mesma raça, todas as características originais da cepa são imediatamente restabelecidas. O mesmo não acontece se a inoculação for feita em cães. Em um de cinco cães, inoculados com apenas 2.000 tripanastigotas, autopsiado na fase aguda da infecção, foi observado miocardite violenta e difusa com gravíssimos fenômenos regressivos das células cardíacas na presença de poucos ninhos de amastigotes. Este fato não foi até então observado em mais de cinquenta cães inoculados com a cepa Be-78 mantidas em laboratório através de passagens sanguíneas sucessivas em camundongos. Curiosamente, a cepa Be-62 de T. cruzi, isolada da mesma paciente 16 anos antes, estudada nas mesmas condições, não apresentou nenhuma mudança significativa em suas características.

Estes dados sugerem que a cepa Be-78 é constituída de sub-populações de T. cruzi cuja expressão é fortemente alterada depois de passar por células “Vero”. Agora, ambas as cepas serão clonadas e caracterizadas através do estudo de Isoenzimas e de DNA para verificar esta hipótese.

Financiado pela FINEP, CNPq e FAPEMIG.
THE COMPLEXITY OF THE CIRCULATION OF Trypanosoma cruzi IN THE NATURAL ENVIRONMENT: BIOLOGICAL AND BIOCHEMICAL CHARACTERIZATION OF MARSUPIAL AND RHODNIUS PROLIUS ISOLATES.

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Going further with our studies on the circulation of Trypanosoma cruzi in marsupials (D. marsupialis and P. opossum) in the natural environment, we determined the isoenzyme and k-DNA patterns as well as the mouse mortality rate of five isolates from naturally infected R. prolirus, four isolates from D. marsupialis and five isolates from P. opossum with natural infections. The marsupials as well as the bugs were captured in the same area (Tequendama, Rio de Janeiro). The 14 isolates could be arranged in five distinct schizodeme patterns. The number of schizodemes of the Philander isolates was higher (four) than of the Didelphis isolates which presented two distinct schizodeme patterns. Didelphis and Philander shared only one schizodeme.

All five isolates derived from R. prolirus displayed exactly the same schizodeme.

Mortality was observed in mice infected with three of the Philander isolates. Four distinct zymodeme pattern were observed among the 14 isolates. The electrophoresis using the enzymes G6PDH, GPI, IDH and ME showed that all but one of the isolates derived from Rhodinus displayed Z1 zymodeme, (Barrett, 1980). The remainder isolate was found to be in both zymodemes 1 and 2 when tested with GPI. All the Philander and the two Didelphis isolates were in the same zymodeme (Z2).

The two other Didelphis isolates displayed Z2 profile when tested with ME and Z1 with the other enzymes.

In experimental conditions we observed that D. marsupialis is a more selective host for T. cruzi than P. opossum which sustain stable infections through Y and C-13 strain (Z2 profiles) as well as through G-49 (Z1 profile). D. marsupialis tend to control and even in some cases, to eliminate infections through Y and C-13 strain (Z2 profile), and maintain stable infections with G-49 strain. It is the first time that we observed opossum naturally infected with a T. cruzi subpopulation with display Z2 profile.

Several authors and also we noticed only Z1 in opossum isolates, Z2 was considered by Barrett, (Trans. Royal Trop. Med. Hyg. 74:8-49) as being essentially related to the domiciliar transmission cycle. It seems that the species of D. marsupialis and P. opossum are inhabiting themselves through distinct mechanisms since all isolates from P. opossum presented the Z2 profile on the contrary to the D. marsupialis and R. prolirus isolates.

Our results evidence that the sylvan transmission cycle of T. cruzi is far more complex than it was assumed and that besides the classical bug-mammal cycle, other infection processes can be responsible for the broad distribution of T. cruzi among syuran mammals. It is worthwhile to emphasize that Z2 profile displayed by one of the bugs isolate when tested for GPI. Heterogeneous profiles have already been described in T. cruzi.

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CHANGES ON THE BEHAVIOUR OF Trypanosoma cruzi POPULATIONS AFTER INFECTION FOR LONG PERIODS OF TIME IN DOGS

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Natural populations of T. cruzi differ in several aspects. Although biological and biochemical approaches have shown differences among strains and clones derived from strains of parasite (Romanha et al., 1979; Morei et al., 1980; Marques de Arojo et al., 1988; Maciel et al., 1992; Breser, 1993). Little is known about the pattern of T. cruzi population to the nature defects of vertebrate hosts during long periods of infection.

To verify this phenomenon comparative studies were performed between Berenice-78 (Be-78) and Colombian original strains with different populations isolated from dogs after different time of infection.

Thus our study was carried out with 3 isolates of Be-78 strain obtained from dogs after 8 years (Be-78A and Be-78B) and 2 years of infection (Be-78C) and 2 isolates of Colombian strain obtained after 8 years (Colombian-A) and 16 years (Colombian-B) of infection in the same experimental model.

Initially biological parameters such as infectivity, parasitemia and mortality rate were studied in Swiss mice inoculated intraperitoneally with 5000 blood trypomastigotes.

No changes were observed in relation to infectivity and mortality of mice inoculated with the original Be-78 strain and their different isolates after 3 successive blood passage in mice. However important variations concerns with pre-patent periods, peak of parasitemia and patent periods of the infection were observed. They were respectively: (1) 6 days, 50,000 trypomastigotes/0.1 ml of blood and 37 days for Be-78; (2) 9 days, 70,000 trypomastigotes/0.1 ml of blood and 47 days for Be-78A; (3) 9 days, 20,000 trypomastigotes/0.1 ml of blood and 47 days for Be-78B; (4) 9 days, 20,000 trypomastigotes/0.1 ml of blood and 34 days for Be-78C isolate.

Both isolates of Colombian strain obtained from dogs (Colombian-A and Colombian-B) showed homogenous behaviour after 10 successive blood passage in mice (peak of parasitemia of 400,000 trypomastigotes/0.1 ml of blood, 0% mortality and patent period of 90 days). However they were very different from the original Colombian strain that presented a peak of parasitemia of 1,000,000 parasites/0.1 ml of blood, 40% of mortality and patent period of 40 days.

These data suggest a decrease in virulence in T. cruzi populations after many years of chronic infection in vertebrate host. Similar results were obtained by Lance et al. (1992) with Be-62 and Be-78 strains isolated from Berenice patient after an interval of 16 years when they were studied in mice and dogs.

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BI-036
CHANGE OF BEHAVIOUR OF TWO STRAINS OF TRYPSANOSOMA CRUZI AFTER CULTIVATION IN VERO CELLS

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Previous studies have shown that two strains of T. cruzi isolated from the Berenice patient, considered the first human case of Chagas' disease (Chagas, 1909), respectively in 1962 (Be-62) and in 1978 (Be-78) are distinct in relation to their biological and biochemical (isoenzymes and restriction endonucleases, K-DNA, profiles) parameters.

Comparative studies of these two strains in Swiss and C3H mice showed remarkable differences between them. Be-62 strain presents a very high parasitemia with peak on the 8th day after infection (DAI) and 100% of mortality until the 20th day. However, Be-78 strain showed a low parasitemia with peak around 12th DAI, patent parasitemia until around day 30 and no mortality during the acute phase of the disease.

We present here data showing that these strains behave differently in mice after cultivation in “vero” cells. Inocula of 50,000 trypomastigotes of both Be-68 and Be-78 strains derived from the 1st, 6th and 12th passages in “vero” cells in Swiss and C3H mice induced an acute phase of the disease more severe than that observed by blood trypomastigote inoculation.

Be-78 strain showed an increased parasitemia ranging about 50 times greater than blood forms infection after the 1st passage in “vero” cells and a rate of mortality of 100% never observed before. With Be-62 strain these alterations were not significant. Mice infected with trypomastigotes of both strains, derived from the 6th and 12th passages in “vero” cells, showed a gradual decrease of parasitemia and mortality.

The histopathological studies with Be-78 strain infections were always compatible with the parasitological findings. A severe pathological damage and parasitism, mainly in the myocardium and skeletal muscle, was also observed. The morphology of the amastigote nests as well as their number and size were always large and different from those observed in mice infected with blood trypomastigotes during successive passages.

These data suggest the occurrence of mixed infection of T. cruzi in the patient Berenice. Both strains are being cloned to verify this hypothesis.

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BI-037
GENETIC EXCHANGE AS POSSIBLE SOURCE OF GENOMIC DIVERSITY IN TRYPSANOSOMA CRUZI.

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Extensive phenotypic and genotypic variation has been reported between populations of T. cruzi, but a clonal population structure supported by significant deviations from the theoretical Hardy-Weinberg equilibrium. In this study, isoenzyme and RAPD analysis of a group of isolates from the Amazon basin of Brazil were characterised as belonging to principal zymodeme 1 (Z1). Two different alleles for the enzyme phosphoglucomutase (EC 2.7.5.1) and PGM were observed together with the corresponding putative heterozygous profile. Evidence of parental and hybrid profiles was also found using RAPD. Biological clones derived from each PGM phenotype group produced PGM and RAPD profiles indistinguishable from those of uncloned isolates. The observed distribution for each PGM phenotype was almost identical to that predicted by the Hardy-Weinberg distribution, suggesting that random mating might occur in this population. This seems to be the first circumstantial evidence of genomic diversity arising as a consequence of genetic exchange in a sylvatic transmission cycle of T. cruzi. Genetic exchange in T. cruzi might have important medical, epidemiological, and taxonomic implications.

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