Trypanosoma cruzi: Variability of stocks from Colombia determined by molecular karyotype and minicircle Southern blot analysis

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Abstract

Nineteen *Trypanosoma cruzi* stocks, most of them of wild origin, and four *Trypanosoma rangeli* stocks from Colombia were analysed by molecular karyotype analysis with cloned DNA cruzipain as the probe. Another 27 cloned stocks of *T. cruzi* from different geographic areas of South America were used as reference for *T. cruzi* lineages. Phenetic analysis of chromosome size polymorphism demonstrated a great variability of Colombian *T. cruzi* stocks, suggesting that most belong to lineage I, although two of them belong to lineage II. The 2 lineage II *T. cruzi*, 17 *T. cruzi* lineage I, and 3 *T. rangeli* stocks from Colombia were studied further by Southern blot analysis with a panel of kinetoplast DNA minicircle probes. Hybridisation results indicate that the two *T. cruzi* II stocks are genetically distant from each other and from *T. cruzi* lineages IIb, IId, and IIe from Chile. Finally, *T. cruzi* minicircle probes do not cross-hybridise in any stringency condition tested with *T. rangeli* minicircles, a clear indication that these parasites can be easily distinguished by this method.

Index Descriptors and Abbreviations: Molecular karyotype; Minicircle probes; *Trypanosoma cruzi*; *Trypanosoma rangeli*; Colombia

1. Introduction

Trypanosoma cruzi is the aetiologic agent of Chagas' disease, which affects approximately 20 million people in Central and South America (WHO, 1997). *T. cruzi* has a broad host range and infects wild and domestic mammals, thus giving rise to a large reservoir of parasites. The parasites are transmitted by several species of blood-sucking reduviid bugs. Population genetic studies of *T. cruzi*, mainly based on genetic markers such as isoenzymes, revealed a high genetic variability (Tibayrenc and Ayala, 1988) and an essentially clonal population structure (Tibayrenc and Ayala, 1993). It is now clear that the *T. cruzi* taxon is composed of two main phylogenetic lineages named as *T. cruzi* I and *T. cruzi* II (Anon., 1999; Miles et al., 1977). *T. cruzi* I (TCI) was originally described from sylvatic cycles in Brazil and corresponds to the previously identified zymodeme Z1 (Brisse et al., 2000; Yeo et al., 2005). This has led to the misconception that TCI is always associated with sylvatic transmission cycles. However, TCI includes parasites circulating in both domestic and sylvatic environments (Brisse et al., 2000; Montilla et al., 2002). In contrast, *T. cruzi* II (TCII) which consists of five sublineages (named IIa–e), the corresponding to Z2 (lineage IIb) plus IId and IIe predominates in domestic environments. Meantime Z3 (lineage IIa) plus IIc predominates in the sylvatic environments (Brisse et al., 2000). Genetic characterisation of *T. cruzi* is not only important for population genetics and phylogenetics, but also for epidemiological purposes. On one hand, it allows exploration of the link between heterogeneity among *T. cruzi* isolates and the pleomorphism in severity and symptoms of the disease that is observed in different geographic regions (Dvorak, 1984; Miles, 1983). On the other hand, in the context of current vector control programmes, it would

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permit tracking *T. cruzi* populations pertaining to the sylvatic cycle, which might constitute a potential reservoir of reinfestation in the domestic transmission cycle (which is specifically targeted by the control programmes).

In Colombia several insect vectors are involved in parasite propagation: *Rhodnius prolixus* in domestic environments, and *Rhodnius pallescens*, *Rhodnius colombiensis*, and *Panstrongylus geniculatus* in wild environments. The first report of genetic heterogeneity of *T. cruzi* was a study using isoenzymes, which showed that in this country most of the *T. cruzi* stocks belong to zymodeme Z1, and some are genetically close to zymodeme Z3 (Saravia et al., 1987). More recent studies combining isoenzyme and schizodeme analyses showed a clear evidence of demic subdivision between Eastern and Western stocks separated by the Andes Mountains and the Magdalena River, which is likely due to the geographic isolation generated by these topographic features (Jaramillo et al., 1999).

In the present study, we focused on insect samples of *T. cruzi* pertaining to the sylvatic cycle, as they constitute potential vectors of reinfestation in the domestic transmission cycle. Two markers were used to characterise parasites: (i) a nuclear marker, the size polymorphism of the cruzipain-bearing chromosome, which clearly differentiates lineages I and II and allows discrimination of stocks within each lineage (Henriksson et al., 2002), and (ii) a kinetoplast marker, the similarity between minicircle molecules as evidenced by DNA hybridisation, which also has fingerprinting properties (Torres et al., 2004). Our sample was also compared with reference *T. cruzi* stocks from the different lineages and with *Trypanosoma rangeli* stocks from Colombia. Results of the two characterisation methods were compared and analysed in an epidemiological context.

2. Materials and methods

2.1. Parasites

Epimastigote forms were grown in LIT medium at 28 °C, harvested by centrifugation at 3000*g* for 10 min and suspended in PBS (Chiari and Camargo, 1984). Table 1 provides information on the geographic area and host origin of the Colombian stocks and reference stocks of *T. cruzi* and *T. rangeli*.

2.2. Size polymorphism of cruzipain-bearing chromosomes

The procedure for preparing intact chromosomal DNA in agarose blocks was described previously (Engman et al., 1987). Chromosomes from *T. cruzi* and *T. rangeli* isolates were resolved in a CHEF apparatus and transferred to a nylon membrane as described elsewhere (Henriksson et al., 2002). A cruzipain probe corresponding to the last 41 amino acids of the central domain and the 131 amino acids of the C-terminal domain of this protein (Campetella et al., 1992) was labelled with ^{32}P by random priming, hybridised and washed under high stringent conditions (Sambrook et al., 1989). Data analysis was performed by calculating the absolute chromosomal size difference index (aCSDI, Dujardin et al., 1995); UPGMA trees based on the aCSDI distance data were constructed using the PHYLIP software package (Felsenstein, 1993).

2.3. kDNA minicircle analysis

Total DNA from *T. cruzi* epimastigotes was obtained by phenol extraction and used as template for PCR amplification of minicircle with primers 121 and 122 as described elsewhere (Solari et al., 2001). PCR products of 330 bp were analysed by electrophoresis on 2% agarose gels, visualised by ethidium bromide staining and transferred to a nylon membrane as described elsewhere (Solari et al., 2001). Seven specific probes corresponding to the Colombian (AF1, AMP05, and STP3.3), and Chilean (sp104 cl1, CBB cl3, v195 cl1, and NR cl3) stocks were made. They were prepared by PCR with the oligonucleotide primers CV1 and CV2, which are directed to the variable regions of the minicircles as described (Veas et al., 1991). The DNA products of 290 bp were digested with the restriction endonucleases *Sau*96I and *Sca*I to remove part of the oligonucleotide primer selected in the conserved region of the minicircle. This procedure therefore generated 250 bp probes, which contain only sequences of the variable region of the minicircles, which were further separated by electrophoresis in low-melting-point agarose and further purified with the Qiagen extraction Kit. After ^{32}P labelling, the probes were hybridised on the membranes mentioned above, using a standard method (Sambrook et al., 1989). However, two different stringency conditions were used in the washing step: 2× SSC, 0.1% SDS at 65 °C (low stringency condition), and later 0.1× SSC, 0.1% SDS at 65 °C (high stringency condition). The interpretation of the hybridisation results after washing in two stringency conditions allowed determination of whether minicircle DNA of genetically close stocks can cross-hybridise.

3. Results

3.1. Size polymorphism of the cruzipain-bearing chromosome

CHEF-resolved chromosomes were hybridised with the cruzipain probe. Most stocks gave a unique hybridisation pattern, but no hybridising bands were detected in the *T. rangeli* stocks studied. Reference stocks were separated into two major clusters (called A and B), corresponding to lineages I and II, with the exception of CANIII (lineage IIa) that clustered with members of lineage I (Fig. 1). Within cluster A, there were three subgroups, while cluster B contained four subgroups in which sublineages IIb–e were dispersed. Colombian isolates were found in three subgroups: (i) the stock LB46 in A2, (ii) most of them in A3 together with Venezuelan reference stock OPS21, and (iii) the stocks AF1 and AMP05 in B3, together with IIb and IIc reference stocks.

Table 1
Biological and geographical origins of *T. cruzi* and *T.rangeli* stocks under study

Stock	Origin locality	Host	Date of collection/classification
T. cruzi	Colombia		
AC16	Acandi, Choco	*Rhodnius pallescens*	1999/I[a]
AC32	Acandi, Choco	*R. pallescens*	1999/I[a]
AF1	Amalfi, Antioquia	*Panstrongylus geniculatus*	1993/II[a]
AMPO5	Amalfi, Antioquia	Dog	1998/II[a]
Cas7	Paz de Ariporo, Casanare	*R. prolixus*	1995/I[a]
Cas16	Talaoho, Casanare	*R. prolixus*	2000/I[a]
Coy8	Coyaima, Tolima	*Didelphis marsupialis*	1991/I
Gal34	Galeras, Sucre	*R. pallescens*	1991/I[a]
LB46	Las Brisas, Sucre	*R. pallescens*	1999/I[a]
LB53	Las Brisas, Sucre	*Triatoma dimidiata*	1999/I[a]
LB54	Las Brisas, Sucre	*T. dimidiata*	1999/I[a]
SB94	San Bernardo, Cordoba	*T. dimidiata*	1999/I[a]
SB1	San Bernardo, Cordoba	*R. pallescens*	1998/I[a]
SB51	San Bernardo, Cordoba	*R. pallescens*	1999/I[a]
SC2	San Carlos, Antioquia	*D. marsupialis*	1989/I
SO22	San Onofre, Sucre	*Eratyrus cuspidatus*	1995/I
Put1	Puerto Asis, Putumayo	*R. robustus*	1992/I[a]
STP3.1	Coyaima, Tolima	*R. colombiensis*	1991/I
STP3.3	Coyaima, Tolima	*R. colombiensis*	1991/I
T. rangeli	Colombia		
SO29 (*T. rangeli*)	San Onofre, Sucre	*R. pallescens*	1995
LDG (*T. rangeli*)	Antioquia	Human	1989
J_1 (*T. rangeli*)	Antioquia	Human	1989
J_2 (*T. rangeli*)	Antioquia	Human	1989
T. cruzi (reference)			
Lineage I			
13379 cl7	Bolivia	Human	Not known/I
SO34 cl4	Bolivia	*T. infestans*	1986/I
P11 cl2	Bolivia	Human	Not known/I
P209 cl1	Bolivia	Human	Not known/I
Esquilo cl1	Brazil	*Sciurus aestuans ingramini*	Not known/I
Gamba cl1	Brazil	*Didelphis azarae*	Not known/I
Cuica cl1	Brazil	*Philander opossum*	Not known/I
Cutia cl4	Brazil	*Dasyprocta agouti*	Not known/I
Sylvio X10 cl1	Brazil	Human	Not known/I
Sylvio X10 cl7	Brazil	Human	Not known/I
SpAI	Chile	*Mepraia spinolai*	Not known/I
LGN cl1	Chile	Human	1980/I
sp104 cl1	Chile	*M. spinolai*	1980/I
OPS22 cl1	Venezuela	*P. geniculatus*	1977/I
OPS21 cl11	Venezuela	Human	1977/I
Lineage II			
SC43 cl1	Bolivia	*T. infestans*	1981/IId
Tu 18 cl2	Bolivia	*T. infestans*	1981/IIb
CAN III cl1	Brazil	Human	1968/IIa
Esmeraldo cl3	Brazil	Human	1971/IIb
v2148 cl1	Brazil	Human	Not known/IId
V2149 cl1	Brazil	*T. infestans*	Not known/IId
CL Brener	Brazil	Human	1963/IIe
M6241 cl6	Brazil	Human	Not known/IIc
Tulahuen FKIIA cl2	Chile	*T. infestans*	Not known/IIe
MN cl2	Chile	Human	Not known/IId
NR cl3	Chile	Human	Not known/IId
CBB cl3	Chile	Human	Not known/IIb
V195 cl1	Chile	*T. infestans*	1992/IIe

[a] Classified in this study.

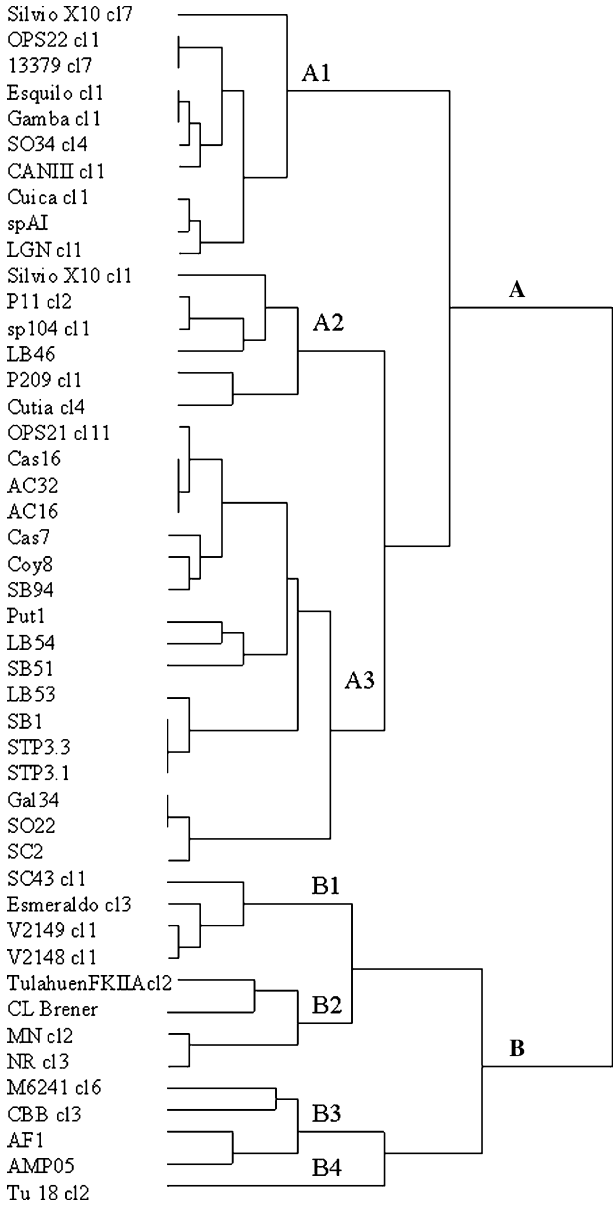

Fig. 1. Dendrogram constructed from absolute chromosomal size difference index (aCSDI). A1–3 and B1–4: names of the clades observed in the aCSDI dendrograms.

3.2. kDNA analysis

A 330-bp kDNA minicircle fragment was amplified in all stocks, except in *T. cruzi* PUT cl1 and in *T. rangeli* stocks (J1, J2, and LDG stocks) which showed a larger amplicon. Reference *T. cruzi* stocks were not studied, except for sp104 cl1, CBB cl3, v195 cl1, and NR cl3. These amplicons were hybridised with each of the seven probes, providing similar results. After low-stringency post-hybridisation washing, a positive hybridisation was observed in all *T. cruzi* amplicons. On the contrary, after high stringency washing, a positive hybridisation was only encountered with amplicons corresponding to the stocks from which the Colombian probes were made and with equivalent stocks when Chilean probes were used (not shown). As a whole, these results suggest that closely related *T. cruzi* stocks present absence of cross-hybridisation between their variable minicircle sequences of kDNA, especially within *T. cruzi* I.

4. Discussion

Two different genetic markers (molecular karyotype and minicircle DNA) were applied to the characterisation of a sample of *T. cruzi* stocks originating essentially from sylvatic habitats in different endemic areas of Colombia, therefore representative of the variety circulating in this country. Both markers revealed a great diversity within present sample. On one hand, the cross-hybridisation of minicircle amplicons with seven kDNA probes suggests that each Colombian *T. cruzi* stock studied is unique, therefore we are unable to found association between *T. cruzi* stocks and particular host. On the other hand, the molecular karyotype using cruzipain as its own probe is very informative, with the only exception that the representative of *T. cruzi* lineage IIa used was classified as *T. cruzi* I. This represents a minor limitation of the method with only one probe. However, this method allows classifying the Colombian parasites into three categories. Fourteen out of 17 stocks without previous information were for the first time genotyped here by molecular karyotype.

The great heterogeneity observed by minicircle hybridisation assays and molecular karyotype patterns is not surprising for *T. cruzi* stocks of Colombia, since similar results have been observed previously using other molecular markers (Jaramillo et al., 1999; Marquez et al., 1998; Montilla et al., 2002; Saravia et al., 1987; Triana et al., 1999). This indicates the genetic heterogeneity of *T. cruzi* in various sylvatic hosts from endemic areas of Colombia, as was observed in Northeast Brazil and French Guiana (Britto et al., 1995; Lewicka et al., 1995). Minicircle kDNA probes and hybridisation tests with genetically related *T. cruzi* clones exhibit an absence of cross-hybridisation, as was also reported with Mexican *T. cruzi* stocks (Bosseno et al., 2000).

Two results deserve particular attention in the context of surveillance and control of Chagas' disease. On one hand, 17 stocks originating from insect vectors of the wild cycle (*R. pallescens* and *R. colombiensis*) clustered with reference strains of lineage I. On the other hand, we found one stock isolated from a domestic dog and other isolated from *P. geniculatus*, which grouped within lineage II, aspect that support the presence of *T. cruzi* II in sylvatic and domestic cycles, as was reported by Montilla et al. (2002). *P. geniculatus* is very widespread in South America and even though it rarely colonizes human dwellings, it is attracted by light, and preferentially transmits *T. cruzi* II (Gaunt and Miles, 2000). Further work is necessary to know if these results are suggestive of the emergence of new transmission cycles, e.g., with adaptation of sylvatic vectors to domestic environments. This question is highly relevant as such phenomena could seriously jeopardise the current Chagas control programmes.

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