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Research brief

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.