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High variability of Colombian *Trypanosoma cruzi* lineage I stocks as revealed by low-stringency single primer-PCR minicircle signatures

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Abstract

In Colombia, high genetic variability has been found among *Trypanosoma cruzi* stocks isolated from different vector and host species, using isoenzyme analysis and RFLP of total kinetoplastid DNA (kDNA), suggesting that several genetically related *T. cruzi* populations might be present within a single geographical area or adjacent ones. The objective of this study was to use the low-stringency single primer (LSSP)-PCR technique on variable regions of kDNA minicircles of *T. cruzi* to determine possible genetic relationships among stocks from distinct geographical regions of Colombia and different vector species and hosts. Although LSSP-PCR analysis showed a high genetic variability among 30 Colombian *T. cruzi* stocks, 29 of them belonged to *T. cruzi* lineage I, confirming that this lineage is predominant in different vector and host species from Colombia. Interestingly, one stock isolated from a *Pastrongylus geniculatus* bug was identified as *T. cruzi* lineage IIb, using PCR strategies targeted to the intergenic region of miniexon genes, a sequence encoding the D7 domain of the 24sα ribosomal genes and the A10 fragment, being this finding, the first description of this lineage in Colombia.

The LSSP-PCR signatures allowed correlation of most isolates with their respective geographical origins, and in one case from host and vector specimens at a same region, suggesting a transmission event. Moreover, variations in LSSP-PCR profiles among *T. cruzi* I stocks from a same region suggest that they may have a multiclonal character. Our results show that LSSP-PCR is a fast, valuable technique for characterization of intra-lineage polymorphism among *T. cruzi* stocks.

Keywords: Chagas; *Trypanosoma cruzi*; LSSP-PCR; Genetic variability; Lineage

1. Introduction

Chagas' disease caused by *Trypanosoma cruzi* is an important public health problem, affecting 16–18 million people in Central and South America. It has been estimated that 1.3 million people in Colombia are infected and 3.6 million are at risk of acquiring the infection (Moncayo, 2003). The severity and symptoms of Chagas' disease vary among different geographical regions and it has been suggested that this variability could be determined by genetic factors, both host- and parasite-related (Macedo and Pena, 1998). Initial characterization of natural populations of *T. cruzi* stocks suggested

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that they can be classified into three different groups or "zymodemes" (Miles et al., 1978). However, high genetic heterogeneity was demonstrated by analysis for size polymorphism of kinetoplast DNA restriction fragments (Morel et al., 1980), DNA fingerprinting (Macedo et al., 1992) and molecular karyotyping (Henriksson et al., 1996).

Long-term clonal evolution together with episodes of genetic exchange led to the individualization of six discrete genetic subdivisions or lineages, designated as *T. cruzi* I, *T. cruzi* IIa, *T. cruzi* IIb, *T. cruzi* IIc, *T. cruzi* IId and *T. cruzi* IIe, which include all typed strains and cloned stocks thus far isolated (Tibayrenc, 2003; Brisse et al., 2003).

T. cruzi lineages appear to be distributed differentially among triatomine and host species and habitats in different geographical areas (Higo et al., 2004; Yeo et al., 2005). Although all *T. cruzi* populations cause the human disease, epidemiological studies suggest that *T. cruzi* IIb, IId and IIe are more related to anthroponotic environments and chronic Chagas' disease patients, *T. cruzi* lineages IIa and IIc to sylvatic environments, and *T. cruzi* lineage I to both (Yeo et al., 2005; Marcet et al., 2006). These host and geographic specificities are associated with different biological properties, such as growth rate, histotropism, antigenicity, pathogenicity, infectivity of potential insect vectors, drug susceptibility, chromosome number, and DNA content, which are likely to represent key determinants in transmission and pathogenesis of Chagas' disease (Macedo and Pena, 1998; Macedo et al., 2004). The identification of lineages, natural isolates and strains of *T. cruzi* have been established in parasite isolates by a plethora of biochemical and molecular markers (Brisse et al., 2001; Macedo et al., 1992; Marcet et al., 2006).

High genetic variability of Colombian *T. cruzi* stocks has been found based on isoenzyme analysis and restriction fragment length polymorphism of kDNA (Saravia et al., 1987; Márquez et al., 1998; Triana et al., 1999; Jaramillo et al., 1999; Montilla et al., 2002). These works showed that *T. cruzi* I (zymodeme I) prevailed in Colombia; however, Saravia et al. (1987), also reported the existence of Zymodeme 3-like stocks and Montilla et al. (2002), the presence of *T. cruzi* II.

Low-stringency single specific primer-polymerase chain reaction (LSSP-PCR) for amplifying the variable region of the *T. cruzi* kDNA minicircles permits translation of the intra-lineage polymorphism in the nucleotide sequence into single reproducible profiles (Vago et al., 1996). Changes in a base may alter the multiband pattern generated by LSSP-PCR, producing new signatures that are diagnostic for specific alterations (Pena et al., 1994; Vago et al., 1996, 2000; Andrade et al., 1999).

The objective of the present study was to use LSSP-PCR of kDNA to determine possible genetic association among *T. cruzi* stocks from a geographical region and to evaluate the usefulness of this technique as a molecular tool for epidemiological analysis of *T. cruzi* stocks isolated from different host and vector species in several geographical regions of Colombia, representing areas of low, medium and high endemicity for Chagas' disease.

2. Material and methods

2.1. Origin of T. cruzi stocks

Thirty Colombian *T. cruzi* stocks were analysed during the present study. These were isolated from different host species and geographical regions of Colombia, representing areas of low, medium and high endemicity for the parasite (Moreno, 1997). The *T. cruzi* II stocks CL Brener, Noel, Y and JGBH from Brazil, Tulahuen from Chile, and Gal57 of *T. rangeli* from Colombia were used as reference strains (Table 1).

2.2. Culture of parasites

Epimastigotes were cultured at 28 °C in LIT medium supplemented with 5% bovine foetal serum. The stocks were subcultured every 7 days during the exponential growth phase (Camargo, 1964).

2.3. Extraction of DNA

Epimastigotes in logarithmic phase were harvested by centrifugation at 3000 rpm and washed three times with PBS at pH 7.4. DNA was extracted by the salting-out method (Miller et al., 1988).

2.4. PCR-based identification of T. cruzi phylogenetic lineages

To distinguish between *T. cruzi* I and *T. cruzi* IIb, IId and IIe phylogenetic lineages, a PCR strategy targeted to the intergenic region of the miniexon genes (SL-DNA PCR) was carried out, using sense oligonucleotides 5-GTGTCCGCCACCTCCTTCGGGCC-3′ (TC1, group II specific), 5′-CCTGCAGGCACACGTGTGTGTG-3′ (TC2, group I specific), and antisense oligonucleotide 5′-CCCCCCTCCCAGGCCACACTG-3′ (TC, common to groups I and II) (Souto et al., 1996). PCR was performed in 25 μl of reaction mixture containing 25 ng of DNA, 50 mM KCl, 10 mM Tris–HCl (pH 8.0), 200 μM