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Trypanosoma cruzi: Sequence analysis of the variable region of kinetoplast minicircles

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Abstract

The comparisons of 170 sequences of kinetoplast DNA minicircle hypervariable region obtained from 19 stocks of *Trypanosoma cruzi* and 2 stocks of *Trypanosoma cruzi marenkellei* showed that only 56% exhibited a significant homology one with other sequences. These sequences could be grouped into homology classes showing no significant sequence similarity with any other homology group. The 44% remaining sequences thus corresponded to unique sequences in our data set. In the DTU I ("Discrete Typing Units") 51% of the sequences were unique. In contrast, in the DTU IId, 87.5% of sequences were distributed into three classes. The results obtained for *T. cruzi marinkellei*, showed that all sequences were unique, without any similarity between them and *T. cruzi* sequences. Analysis of palindromes in all sequence sets show high frequency of the *Eco*RI site. Analysis of repetitive sequences suggested a common ancestral origin of the kDNA. The editing mechanism that occurs in kinetoplastidae is discussed.

Index Descriptors and Abbreviations: *Trypanosoma cruzi*; Minicircles; Kinetoplast DNA (kDNA) polymorphism; Hypervariable domain; DTU (Discrete Typing Units); Evolution; Editing

1. Introduction

Chagas' disease is a parasitosis caused by *Trypanosoma cruzi*, a flagellate protozoan of the Kinetoplastidae family, transmitted by hematophagous insects of the Reduviidae family and the Triatominae subfamily. This disease is a major public health problem in Latin America, affecting about 14 million people with 100 million people being at risk. Chagas' disease shows considerable clinical variability (cardiac, digestive, or cardio-digestive) but the reason for this is not known. Evidence nevertheless suggests that both host and parasite factors may be involved (Buscaglia and Di Noia, 2003; Risso et al., 2004). Deciphering the population genetic structure of *T. cruzi* is thus of the utmost importance for a better understanding of the pathogenesis of Chagas' disease. Studies based on isoenzyme analysis, riboprinting analysis, RNA promoter activity, analysis of mini-exon gene sequences and microsatellite markers have provided clear evidence that *T. cruzi* consists of two highly divergent genetic subgroups, each considerably heterogeneous (Tibayrenc, 1995; Souto et al., 1996). They were designated lineages *T. cruzi* I and *T. cruzi* II by a college of experts (Tibayrenc, 1995; Anon, 1999). These lineages cannot be equated to clades since some horizontal gene transfer played a notable role in their origin (Bogliolo et al., 1996; Gaunt et al., 2003). This led Tibayrenc to propose the descriptive concept of Discrete Typing Unit (DTU) to

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account for sets of stocks genetically closer to each other than to any other stock, and identifiable by common sets of genetic markers called "tags" (Tibayrenc, 1998). Lineages 1 and 2 (Momen, 1999) can thus be equated to such DTUs, DTU I, and DTU II respectively.

Multilocus enzyme electrophoresis and random amplified polymorphic DNA data have shown that DTU II can be separated into five smaller lineages, namely DTU II a–e (Barnabé et al., 2000; Brisse et al., 2000), which can also be characterized by their gene expression profile. Furthermore, parity between phylogenies obtained from RAPD and MLEE polymorphisms and sequence polymorphism of expressed genes was demonstrated (Telleria et al., 2004). *T. cruzi*, alike other members of the Trypanosomatidae family, possesses a unique mitochondrion, the kinetoplast, that extends throughout the cell. Kinetoplast DNA represents 20–25% of the parasite's total DNA and consists of a network of circular molecules, maxicircles (25–50 copies per cell) and minicircles (20,000–30,000 copies per cell)(Degrave et al., 1988). Minicircle DNA RFLP experiments (schizodeme analysis) revealed a considerable polymorphism of these molecules (Morel et al., 1980), significantly correlated to isoenzyme diversity (Tibayrenc and Ayala, 1987). The sequencing of some minicircles demonstrated that the sequence (1440 bp) is organized into four equidistant regions of 100 bp with highly similar sequence and an equal number of hypervariable regions. Each variable sequence in one given minicircle appears to be unique (Gonzalez, 1986; Macina et al., 1986; Degrave et al., 1988).

Using primers corresponding to the invariable domains, minicircles were amplified by PCR for a variety of *T. cruzi* isolates and the amplicons were used in reciprocal hybridization experiments. Positive hybridizations were observed only with amplicons from isolates of the same group of stocks, formerly called "clonet" (Veas et al., 1990; Brenière et al., 1992). This approach was used for typing *T. cruzi* stocks in Bolivian and Chilean patients (Brenière et al., 2002; Solari et al., 2001). The clonet specificity of this hybridization suggests that the variable sequences are sufficiently conserved (as a group) within one DTU but vary markedly from one DTU to another. However, so far, only a limited number of these variable sequences has been published.

The amount of genetic information contained in the maxicircle DNA is comparable to that found in the mitochondrial DNA of other eukaryotic cells (Shapiro and Englund, 1995; Shlomai, 2004). The maxicircle contains two rRNA genes and up to 18 structural genes encoding subunits of enzymes associated with the respiratory pathway such as cytochrome-*c*-oxidase subunit II and III (COII and COIII), NADH deshydrogenase subunit 7 (ND7) or ATPase subunit 6 (A6). Transcripts of the maxicircles of kinetoplastid protozoa cannot readily be translated since they contain a variable number of frameshift that must be corrected (edited) for translation to occur. RNA editing consists in the insertion or deletion of uridines in maxicircle DNA transcripts. Small guide RNAs (gRNA) specify the edited sequence, which is complementary to the gRNAs by a G::U, in addition to the Watson–Crick base pairing (for review (Stuart et al., 1997)). Guide RNAs are generally encoded by the minicircles of kinetoplast DNA with some exception however (Kim et al., 1994; Golden and Hajduk, 2005). In all trypanosomatids, a conserved 12 nucleotide region (GGGGTTGGTGTA) provides a relative position and polarity marker for the gRNA genes (Ray, 1989), which are localized between 60 and 100 bp downstream of this region (Avila and Simpson, 1995; Simpson, 1997). The sequences and the role of minicircle-encoded gRNAs in the editing of *T. cruzi* MURF4 gene and ATPase subunit 6 genes were investigated in detail (Avila and Simpson, 1995; Ochs et al., 1996).

However even within all these reports, only a limited number of variable sequences from only few strains have so far been described and recent review on minicircles organization in *T. cruzi* concluded that no group clustering of available minicircle sequences was observed (Junqueira et al., 2005).

In the present work, we report the sequences of minicircle variable regions of 19 *T. cruzi* stocks to evaluate the relationships between this parasite's population structure and kDNA polymorphism.

2. Materials and methods

2.1. Trypanosoma cruzi stocks and culture conditions

The 19 *T. cruzi* stocks selected for this study are representative of the eco-epidemiological genetic diversity of *T. cruzi* (Table 1). They include stocks belonging to four main subdivisions of the species, DTU I, IIb, IId, and IIe. In each of these, 6 stocks were selected. Additionally we used two stocks of the sub-species *T. cruzi marinkellei* as an outgroup reference. All stocks have previously been characterized by MLEE and RAPD (Barnabé et al., 2000; Brisse et al., 2000). The stocks were laboratory-cloned by micromanipulation under microscope control and cultivated in RPMI-1640 (SVF 20%, glutamine 1%). The identity of each strain was ascertained by isoenzymatic markers just prior to the experiments.

2.2. Total DNA extraction

Cultured parasites were harvested to obtain 30 mg parasite cell pellet corresponding to 5×10^6 to 5×10^7 cells. Total DNA was then extracted through the following three steps: (i) lysis of parasite cells (ii) deproteinisation, and (iii) DNA precipitation. DNA quality was verified by electrophoresis in 1.2% agarose gel, and its concentration and purity was measured by spectrometry at 260 nm.

2.3. Amplification of kDNA minicircle variable regions

Oligodeoxynucleotide primers chosen for the amplification of the variable regions in kDNA minicircles were