

## Characterization of *Trypanosoma cruzi*

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The *Trypanosoma cruzi* is a heterogeneous population, composed by a pool of strains which circulate in both the domestic and sylvatic life cycles including humans, vectors and animal reservoirs. Isolation and study of *T. cruzi* populations from different origins demonstrated the presence of a large range of strains with distinct biological, immunological, biochemical and pharmacological characteristics. Studies using cloned or uncloned *T. cruzi* populations, reinforce the heterogeneity of the parasite and demonstrate that the strains are composed of subpopulations with distinct characteristics (Postan et al. 1986, Finley & Dvorak 1987).

Morphological differences in the *T. cruzi* blood forms have already been described by Chagas in 1909 in his classical paper on the discovery of Chagas disease (Chagas 1909). The role of the slender and broad bloodstream forms trypomastigotes were later thoroughly studied by Brener (1973). Nussenzweig et al. (1963) and Nussenzweig and Goble (1966) described immunological differences among *T. cruzi* strains. The strains were divided into three groups according to their cross-reaction with heterologous serum from infected mice. Following these biological and immunological observations, new approaches have been used for molecular characterization of *T. cruzi*.

Enzyme electrophoresis studies have demonstrated distinct *T. cruzi* populations (zymodemes) circulating in the domestic and sylvatic transmission cycles and provided a good epidemiological marker for Chagas disease. Miles et al. (1977, 1978) studying *T. cruzi* strains isolated from the Brazilian states of Bahia and Pará, described the

presence of three zymodemes (Z1, Z2 and Z3). Z1 and Z3 parasites were found in the sylvatic cycle and in a few human acute cases whereas Z2 parasites were found restricted in the domestic cycle of transmission. Romanha (1982) and Carneiro et al. (1990) characterizing *T. cruzi* samples isolated from chronic chagasic patients from, the endemic area of Bambuí, Minas Gerais, observed the presence of four distinct zymodemes (ZA, ZB, ZC and ZD). ZA was the same as Z2. ZB showed a characteristic heterozygous pattern generated by the hybridization between the parents ZA and ZC. Thus, in Brazil at least six major *T. cruzi* isoenzyme groups (Z1, Z2 or ZA, Z3, ZB, ZC and ZD) have been reported. Tibayrenc et al. (1986) and Tibayrenc and Ayala (1988), analyzing the isoenzymatic profiles (15 gene loci) of 645 *T. cruzi* samples isolated from a variety of vertebrates and invertebrates hosts with a wide geographical distribution, observed a high genetic variability. They identified at least 43 distinct natural isoenzyme strains (zymodemes or "clonets"). Due to this high genetic variability and the findings of the same "clonets" geographically distant, the authors proposed a multiclonal population structure for *T. cruzi* and that its sexual reproduction was rare or absent (Tybayrenc & Ayala 1988, Zhang et al. 1988).

Besides the isoenzymes, other molecular approaches have also pointed towards a high genetic variability in *T. cruzi* (reviewed by Macedo & Pena 1998). The restriction fragment length polymorphism (RFLP) of the kinetoplast DNA (kDNA) minicircles demonstrated that almost every strain presents a different "schizodeme" (Morel et al. 1980). The heterogeneity was even higher when clones from the same strain were analyzed (Gonçalves et al. 1984). Improvements in the original protocol were made by Sturm et al. (1989) and Ávila et al. (1990). The variable segment of kDNA was amplified by the polymerase chain reaction (PCR) and its RFLP determined. Recently, the sequence variability of this fragment was studied directly in infected mice and chronic chagasic patients tissues, by LSSP-PCR (low-stringency single specific primer) (Vago et al. 1996 a, b). The poly-

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morphism of the nuclear DNA (DNA fingerprinting) showed a good correlation with the schizodeme analysis (Macedo et al. 1992). Randomly amplified polymorphic DNA (RAPD) has also been used as an approach for the analysis of genetic variation and identification of genetic markers. Steindel et al. (1993) and Tibayrenc et al. (1993) observed a direct correlation between RAPD and isoenzyme profiles in *T. cruzi*. Recently, the PCR variant, SSR-PCR (single sequence repeat anchored primer PCR) was applied to study the genetic variability in *T. cruzi* (Oliveira et al. 1997). A hypervariable multiband profile was obtained with the DNA amplification of *T. cruzi* using the (CA)<sub>8</sub>RY primer. The phylogenetic analysis of *T. cruzi* based on eight polymorphic loci of microsatellites (CA)<sub>8</sub> revealed a great genetic distance among the strains (Oliveira et al. 1998). Other nuclear markers as genes that code the ribosomal RNA 24S $\alpha$  and the mini-exon (Souto et al. 1996), spliced leader RNA and rRNA gene promoters (Nunes et al. 1997), have divided *T. cruzi* strains into two major phylogenetic lineages. An association of lineage 1 with domestic and lineage 2 with sylvatic cycle was observed (Zingales et al. 1998). Furthermore these genotypic markers showed a direct correlation with the phenotype characteristics previously determined by the isoenzymes. Restriction fragment length polymorphism of the rRNA gene 18S, allowed the classification of *T. cruzi* strains into three distinct groups, denominated "ribodemes" I, II and III (Clark & Pung 1994). A correlation between the genetic markers of ribosomal RNA subunits 18 S and 24 S was observed.

The lack of condensed chromosomes during cell division, have prevented the *T. cruzi* characterization at the cytogenetic level. However, through pulse-field gel electrophoresis (PFGE) it was possible to verify that the *T. cruzi* genome is organized in approximately 20-25 chromosomal bands ranging from 0.3 to 1.6 Mb, usually with chromosomes larger than 1.6 Mb accumulating in the compression region (Henriksson et al. 1990). The total and nuclear DNA contents have been reported to vary considerably among different *T. cruzi* strains and even between clones of the same strain (Dvorak et al. 1982, McDaniel & Dvorak 1993). A correlation between karyotype pattern and isoenzyme classification has been proposed by Henriksson et al. (1993).

Previous studies on isoenzymes (Miles et al. 1977, Bogliolo et al. 1986, 1996, Tibayrenc et al. 1986), total DNA content (Borst et al. 1982), molecular karyotypes and restriction fragment length polymorphisms (Gibson & Miles 1986, Aymerich & Goldenberg 1986, Henriksson et al. 1990, Dietrich et al. 1990) and more recently microsatellites stud-

ies (Oliveira et al. 1998) are in agreement with diploidy in *T. cruzi* and also support that the genome of this parasite is remarkably plastic. Despite the genetic diversity and diploidy of *T. cruzi*, it has been proposed that this parasite has a clonal population structure and asexual reproduction, this is supported by the great deviation from Hardy-Weinberg equilibrium observed in natural populations (Tibayrenc et al. 1990). Nevertheless, isoenzyme analysis and RFLP of three glycolytic genes demonstrated the presence of the heterozygotes and the corresponding homozygotes circulating in the same area (Bogliolo et al. 1996). These findings support the hypothesis of genetic exchange in *T. cruzi*. In agreement with these results, Carrasco et al. (1996) analyzing isoenzyme and RAPD profiles of *T. cruzi* strains from Central and South America, suggested that genetic exchange occurs during sylvatic transmission of *T. cruzi*, and that it contributes to the generation of phenotypic and genotypic diversity in this parasite.

The correlation between *T. cruzi* genetic structure and the clinical forms of the Chagas disease, as well biological characteristics, as virulence, pathogenicity and susceptibility to drugs, have been extensively investigated by several authors. Although initial studies by Miles et al. (1981) on *T. cruzi* strains from Venezuela and Brazil suggested the possibility of some correlation between zymodemes and clinical forms, later Apt et al. (1987) and Breniere et al. (1989), did not report any relationship between these parameters. In Argentina, Montamat et al. (1996), observed a correlation between Z12 parasites and high incidence of cardiac lesions in chagasic patients, whereas patients with Z1 parasites were likely to remain asymptomatic for a long time. An extensive study of the biological characteristics of 138 *T. cruzi* strains and the histopathological profile in experimental animals permitted the division of the strains into three types or biodemes (Andrade & Magalhães 1997). The authors observed a correspondence between biodemes and zymodemes. A recent study 45 *T. cruzi* strains susceptible and naturally resistant to benznidazole and nifurtimox were analyzed for different molecular markers. The heterozygous profile, zymodeme B, contained exclusively susceptible strains, and occurred predominantly in geographic areas where clinical treatment of Chagas disease has been reported as more effective (Murta et al. 1998).

In conclusion, the molecular markers described, (i) reinforced the populacional heterogeneity in *T. cruzi*, (ii) permitted the division of the *T. cruzi* strains into two groups or lineages, one circulating in the sylvatic cycle and another in the domestic cycle of transmission, (iii) evidenced diploidy and

the genetic exchange in *T. cruzi*, and (iv) in a certain extent, could be associated with drug susceptibility phenotype in this parasite.

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