# *Trypanosoma cruzi:* Clonal Structure of Parasite Strains and the Importance of Principal Clones

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*Trypanosoma cruzi* strains, are complex multiclonal populations that differ in their genetic and biological characteristics and in their behavior in the vertebrate host. *T. cruzi* strains represent subspecies, based on intrinsic characters as antigenic composition (Andrade et al. 1981) susceptibility to chemotherapy (Andrade et al. 1975, Brener et al. 1976), isoenzyme patterns ( Miles et al. 1980, Andrade et al. 1983, Tibayrenc & Ayala 1988) and genomic profils of kDNA (Morel et al. 1980, Avila et al. 1990). Studies on the host-parasite relationships have disclosed marked differences in the determinism of tissue lesions, dependent on tropism, virulence and pathogenicity of *T. cruzi* strains (Andrade et al. 1970, 1985). An extensive study on the biological characteristics of the natural strains and histopathological profile in experimental animals, led to grouping them since 1970 (Andrade et al.), into a few well defined types or biodemes: Types I, II and III (Andrade et al. 1970, 1985, 1997). This biological classification has been recommended by WHO (1986). A possible genetic basis for the biological behavior of the three strain types has been searched at a phenotypic level, by isoenzymic analysis of the prototypes, and other representative strains, isolated from different geographical areas (Andrade et al. 1983). The enzymes PGM (phosphoglucomutase), GPI (glucosephosphate isomerase), ALAT (alanine amino transferase), ASAT (aspartate aminotransferase), allowed a good discrimination between the three strain types and a clear correlation with biological data. By studying 15 gene loci coding for enzymes, in stocks of *T. cruzi* from different geographical areas, Tibayrenc and Ayala (1988), have suggested the possibility of a clonal structure for *T. cruzi* strains, based on the existence of clonal lines without sexual interactions, separated by a long evolutionary process. This suggested a possible correlation between biochemical classification and biological properties. According to those authors, *T. cruzi* strains are natural clones and the natural selection, favouring only certain genetic pattern or combinations, could account for a limited number of isoenzyme strains, represented by three major clones. By using several parameters *in vitro*, Revollo et al. (1998) has confirmed the hypothesis of a correlation between biological and phylogenetic variability of *T. cruzi*, by multilocus enzyme electrophoresis (MLEE), and random amplification of polymorphic DNA (RAPD), based on the genetic distances. Biological types (biodemes), are correlated with the zymodemes which were described by Miles et al. (1980), with the exception of the Type I strain that presented a peculiar electrophoretic profil not described previously (Andrade et al. 1983) and that has been subsequently identified as Z2b; biodeme Type II corresponds to Z2, and Type III, to Z1.

In a survey of 138 strains isolated from different hosts, in different geographical areas of Brazil and other countries of Central and South America, the ubiquitary distribution of the three biodemes was evident (Andrade & Magalhães 1997). However, the exclusive presence of Type II, Z2 strains in the endemic areas of São Felipe and Mambaí was patent, and the predominance of Type I, Z2b in several South America countries became evident, as well as the correlation of Type III, ZI strains with a sylvatic origin. The stability of the biological and isoenzymic characteristics of strains isolated from the endemic area of São Felipe, BA, all of them included into the Type II, Z2, points to the presence of "principal clones" in this area (Andrade 1974, Andrade et al. 1983).

An investigation was performed to stablish the characteristics of the clonal populations of the 21 SF strain, one of the prototypes of the several strains from São Felipe, which was isolated from a patient during the acute phase of the disease. It has been maintained in laboratory by successive passages in mice for more than 15 years. Cloning has

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been performed by isolating one single parasite from peripheral mouse blood and injecting it intraperitoneally into one suckling mouse. From each positive animal, thus representing one clone, inoculum was individually obtained and injected into suckling mice. Five clones and 14 subclones have been obtained and submitted to biological, biochemical and genetic characterization (Campos & Andrade 1996, Campos et al. 1999). Biological and biochemical characterization have shown that all the clones and subclones revealed parasitemic profils characteristics for Type II strains, and similar to the parental strain (21SF), in the infected mice (Campos & Andrade 1996). The biochemical analysis for the enzymes ALAT, ASAT, PGM and GPI, have shown the electrophoretic Z2 patterns, similar to the parental strain. Biological and isozymic homogeneity of the 5 clones and 14 subclones of the 21 SF strain seems to indicate that this *T. cruzi* strain is composed predominantly by similar populations considering their phenotypic characteristics. However, as registered by McDaniel and Dvorak (1993), clones with the same isoenzymic profils, can differ in their schizodemes. Considering the stability of the 21 SF strain and the homogeneity of the biological and isoenzymic characters of its clones and subclones, the genetic profil of this clonal population was then investigated. A comparative analysis by polymerase chain reaction (PCR) amplified minicircles of kinetoplast DNA, of the several clones and subclones, was performed with the objective of clarifying their genomic profil (Campos et al. 1999). Schizodeme was established by comparative study of the fragments obtained from digestion of the 330 bp fragments amplified by PCR, from the variable regions of the minicircles and digested by restriction endonucleases Rsa I and Hinf I. The results have shown a high percentual of similarity between the restriction fragment lenght polymorphism (RFLP) for the parental strain and its clones and among these individual clones and their subclones, at a level of 80 to 100%. This homology indicates the predominance of the same "principal clone" in the 21 SF strain. These results suggest the possibility that *T. cruzi* strains with similar biological and isoenzymic patterns, circulating in the endemic area of São Felipe, are representative of one dominant clone. Well adapted clones can predominate and be selected by environmental conditions and circulate in different areas (Tibayrenc & Ayala 1988). The presence of a dominant clone in the strains isolated from one endemic area, revealed by biological, biochemical and genetic parameters, could be responsible for a predominant tropism of the parasites. This, certainly, may contribute to the pattern of clinico-pathological manifestations, in one geographical area. Macedo and Pena (1998) suggests that multiclonal strains are formed by clonally propagating organisms with different tissue tropisms, correlated with complementary molecular interactions between the invading clones of *T. cruzi* and the host tissues. The biological and biochemical characteristics of *T. cruzi* strains are correlated with different tissue lesions, as first observed in acute infection of mice with strains of different types (Andrade 1985). During the chronic phase, a clearcut influence of the biological type of strain on the histopathological lesions has also been detected. The Type III strains (Z1) were the most pathogenic, determining intense cardiac and skeletal muscle lesions, with patent tissue parasitism, even at the late stage of infection. During the chronic phase, cardiac lesions also occurred in the mice infected with Types I and II strains (Andrade 1990). Besides cardiac lesions, these two types of strain determined significant involvement of the neuronal cells of the myoenteric plexus. Although segmentar inflammatory alterations, in the ganglionic cells, has been detected with the three types of strains, they are more destructive for the neuronal cells, in Y strain (Type I) infections (Souza et al. 1996). The Zymodeme 2 has been identified by Lauria-Pires and Teixeira (1996) in one *T. cruzi* stock from a patient with the digestive form of Chagas disease, and several clones, suggesting the participation of this zymodeme, corresponding to Type II strain, in the pathogenesis of megasyndromes in Chagas disease. Histopathological evidences that cardiac lesions occurred with the three types of strains, correlates well with the fact that a chronic cardiopathy is the main manifestion of human Chagas disease, anywhere this parasitic disease is endemic.

Correlations between the sensibility to benznidazole and nifurtimox and the genetic distances of *T. cruzi* stocks, have been described by Revollo et al. (1998). This is in agreement with the observation that resistance to chemotherapy is related to the biological characteristics of the *T. cruzi* strains (Andrade et al. 1985) . The prototype of Type III strain, the Colombian strain, which is highly resistant to chemotherapy, has recently been cloned, showing an uniformity of biological and isoenzymic characters of its clones. Also it has maintained resistance to chemotherapy, thus confirming the hypothesis that a stable strain represents a predominant clonal population. In the endemic area of Montalvânia, MG, were an overlap between the sylvatic and domestic populations of triatominae has occurred (Luquetti et al. 1986), Types II and III strains have been identified (Andrade et al. 1992); clinical results of treatment with benznidazole and nifurtimox and the response

to treatment in mice infected with these strains, that have been isolated from the same patients of this area, revealed 82% of coincidence (Andrade et al. 1992).

The identification of different biological types, and the genetic confirmation of the existence of populations with significant genetic distances, leads to the conclusion that "principal clones", circulating in one endemic area can be responsible for the disease manifestations and the type of chemotherapic responses seen in patients of these areas.

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