Reflections on the Population Dynamics of *Trypanosoma cruzi*: Heterogeneity Versus Plasticity

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Trypanosoma cruzi, the etiological agent of Chagas disease, exists in nature as a complex of heterogeneous populations. There is a wide spectrum of human disease manifestations (Z Brener 1987 *Mem Inst Oswaldo Cruz 82*: 205-212) and the crucial problem in the study of Chagas disease is a lack of understanding of the factors involved in its pathology. The imposition of natural and artificial pressures can result in the selection of a subset of the population. The marked heterogeneity of *T. cruzi* leads to a search for a relationship between functional parameters of parasites isolated from patients or specific geographical areas and the manifestation of the disease itself. Another crucial, and someway, unsolved question is the influence of this heterogeneity on the stability of the parasite population (JA Dvorak 1984 *J Cell Biochem 24*: 357-371, RW Finley & JA Dvorak 1987 *J Protozool 34*: 409-415).

In essence, the fundamental problems associated with the attempts to understanding Chagas disease were reported by Carlos Chagas in 1909 (*Mem Int Oswaldo Cruz 1*: 159-218) and most of the phenomena currently under study were identified by himself. As the disease can be manifested in different clinical forms from symptomless to an acute fulminate infection or to severe or even inapparent chronic infection, FL Lambrecht (1965 *Rev Inst Med Trop São Paulo 7*: 346-352) advanced the premise that *T. cruzi* may be composed of a genetically heterogeneous population of parasites and that such heterogeneity may be one of the factors that modulate the disease process. In fact, differences in isolates or strains from wide geographical and host ranges have already been well documented (Z Brener 1965 *Ann Trop Med Parasitol 59*: 19-26, SG Andrade et al. 1970 *Gaz Med Bahia 1*: 32-42, Z Brener 1980 *Advances Parasitol 18*: 247-292, WE Gutteridge 1981 *Trans R Soc Trop Med Hyg 75*: 484-492).

With the argument of population heterogeneity in mind, some years ago, researchers from Brazil and outside begun to develop new biological and biochemical approaches to investigate this intriguing parasite. Restriction analyses of kDNA (CM Morel et al 1980 *Proc Natl Acad Sci USA 77*: 6810-6814) have shown a considerable degree of heterogeneity in natural *T. cruzi* populations and important findings such as the occurrence of mixed infection in human, reinfection and selection of subpopulations have been reported (CM Morel 1984 *TDR Series 5*: 333-375, MP Deane et al. 1984 *J Protozool 31*: 276-280, CM Morel et al. 1986 *Parasitol Today 2:* 97-101). Isoenzymatic analyses also confirmed the heterogeneity although initial studies revealed discrete variability showing three or four principal zymodemes (MA Miles et al. 1977 *Trans R Soc Trop Med Hyg 71*: 217-225, 1980 *Trans R Soc Trop Med Hyg 74*: 221-237, AJ Romanha 1979 *Comp Biochem Physiol 62*: 139-142). In recent times, new concepts, models and methods have been introduced to elucidate the complexity of the population dynamics of *T. cruzi*. In 1986, Tibayrenc and co-workers (*Proc Natl Acad Sci USA 83*: 115-119) have proposed that natural populations of *T. cruzi* have a complex multiclonal structure. Although *T. cruzi* is diploid (Tibayrenc *loc. cit*, WC Gibson & MA Miles 1986 *EMBO J 5*: 1299-1305) its reproduction is primarily clonal and sexual recombination is rare or even absent. An extensive zymodeme diversity among distinct clones was discovered indicating that they could hardly be clustered in a few groups (M Tibayrenc & FJ Ayala 1988 *Evolution 42*: 277-292). The term clonet (M Tibayrenc 1990 *Proc Natl Acad Sci USA 87*: 2414-2418) was introduced to explain the intragroup or infraspecific variation. On that account, how many relevant phylogenetic subdivision are there? (M Tibayrenc 1995 *Adv Parasitol 36*: 47-115, RP Souto et al. 1996 *Mol Biochem Parasitol 83*: 141-152). It seems that there is an

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"harmony" between scientists and methodologies in investigating genetic diversity at nuclear and/or mitochondrial DNA levels and heterogeneity is still being reported. On the other hand, evidence supporting genetic exchange in sylvatic *T. cruzi* populations (HJ Carrasco et al. 1996 *Am J Trop Med Hyg 54*: 418-424) and the possibility of occurrence of homozygotes and heterozygotes in sympatric clinical isolates (AR Bogliolo et al. 1996 *Acta Tropica 61*: 31-40) may also explain such diversity. Even in spite of questions on distinct molecular clocks or synapomorphic characters of the molecular markers, polymorphisms are being detected in one or both of the nuclear or kinetoplast genomes (JL Affranchino et al. 1986 *J Protozool 33*: 503-507, M Tibayrenc & FJ Ayala 1987 *CR Acad Sci* Paris *304*: 89-92, A Solari et al. 1992 *Exp Parasitol 75*: 187-195, AM Macedo et al. 1992 *Mol Biochem Parasitol 55*: 147-154, J Henriksson et al. 1993 *Exp Parasitol 77*: 334-348) and correlated with biological features (SG Andrade & JB Magalhães 1997 *Rev Soc Bras Med Trop 30*: 27-35, M Basselin et al. 1998 *Acta Trop 70*: 43-61). At this point two questions arise. Are the populations (or clones) of *T. cruzi* evolving toward increasing or decreasing heterogeneity? Is there a parallel evolution of the nuclear and kinetoplast genomes or a possible interaction between both elements in the same cell? Here the concept of plasticity, as a whole, (biological and genetic) must also be borne in mind. The capacity to adapt to new environments and hosts reflects plasticity. The ability of *T. cruzi* in undergoing its life cycle in the anal glands of the opossun (MP Deane et al. 1984 *Mem Inst Oswaldo Cruz 79*: 513-515, MP Deane 1986 *Parasitol Today 2*: 146-147) is indeed a lesson in versatility. Under stress conditions or selective pressures, chromosomes and minicircles have shown evidence of genetic plasticity in *T. cruzi* and *Leishmania* (JP McDaniel & JA Dvorak 1993 *Mol Biochem Parasitol 57*: 213-222, AMB Alves et al. 1994 *J Euk Microbiol 41*: 415-419, RS Pacheco et al. 1995 *Mol Biochem Parasitol 69*: 197-209). In other words, the nuclear trans splicing (PW Laird 1989 *Trends Genet 5*: 204-208) and the mitochondrial RNA editing (R Benne 1989 *Biochim Biophys Acta 107*: 131-139) mechanisms could, at least in theory, account for an output of alternative proteins or virulence factors. Would such mechanisms be archaic or adapted?

The fundamental question that emerges from this puzzling scene is related to the host-parasite relationship. If the biological entity that evolves is the clonal lineage (FJ Ayala 1993 *Biol Res 26*: 47-63) one can imagine clones with different biological behaviors being reshuffled among the whole circulating population sharing different virulence factors or striving for selective advantages such as distinct growth rates and tropisms. Consequently, are the different genetic characteristics of clones as important as the genetics of the host during the course of the infection? We believe so. As stressed by J Alvar (1994 *Parasitol Today 10*: 160-163) the phenomenum of parasite persistence can be the rule instead of the exception and the reactivation of dormant *T. cruzi* with unusual clinical pictures and different tropism corroborates this hypothesis (D Gluckstein et al. 1992 *Am J Med 92*: 429-432, JCP Amato et al. 1997 *Rev Soc Bras Med Trop 30*: 61-63, RS Pacheco et al. 1998 *Mem Inst Oswaldo Cruz 93*: 165-169). The discovery of distinct tropisms of *T. cruzi* lineages in mice [G Vianna 1911 *Mem Inst Oswaldo Cruz 3*: 1926, RC Melo & Z Brener 1978 *J Parasitol 64*: 475-482, LO Andrade et al. 1997 *Mem Inst Oswaldo Cruz 92* (Suppl. 1): 258] provides support to the hypothesis that the genetic characteristics of the clones are relevant to the pathogenesis of Chagas disease. The recently proposed clonal-histoptropic model (AM Macedo & SDJ Pena 1998 *Parasitol Today 14*: 119-124) associated with direct studies of human infected tissues (AR Vago et al 1996 *Am J Pathol 149*: 2153-2159) will probably contribute a new perceptive regarding clonal heterogeneity and plasticity.

A brief comment, based on our own investigation, that sustains opinions such as clonal heterogeneity, selection of subpopulation and plasticity [Pacheco et al. 1995 *loc. cit.*, CMM Brito et al. 1996 *Mem Inst Oswaldo Cruz 91* (Suppl. I): 278, Pacheco et al. 1998 *loc. cit.*] should also be add to the roll of contributions already referred to, in this text. We have been involved in studying parasite population in two systems and in correlating the biological and/or epidemiological impact in both. In one system (animal model), the exogenous sources of variability (reinfection or accumulation of multiple independent infections) are controlled and the heterogeneity eliminated by cloning and subcloning the initial inoculum. Our results revealed evidence that polymorphisms in minicircles sequences can emerge during infection with a single clone but also can be reverted to the original pattern. Such genetic plasticity may reflect a shift in specific classes of minicircles resulting from RNA editing. The participation of selection in the transition from one profile to another or whether the transition is linked to a change in virulence requires further study. In the other system (patient from endemic area) the exogenous and endogenous source of heterogeneity are not controlled. The study of an uncloned *T. cruzi* isolate from a case of reactivation in a patient with Chagas disease/Aids co-infection showed that the patient was infected with, at least, three distinct parasite subpopulations. One of the subpopulations,

with marked feno and genotypic differences, was used to infect mice. Interestingly, a distinct genotypic profile, but similar to one of the subpopulations from the patient, emerged during the reactivation of the disease after immunosupression of the chronic chagasic mice (CMM Brito MSc Thesis in progress). The patient presented four cerebral lesions and died of intracranial bleeding but neither histopathologic analysis nor the brain specimen culture were carried out due to the non-authorization of his family. During the reactivation, the mice under study also presented meningoencephalitis. The next step is obvious.

As final commentary we shall introduce the elegant vision of Keith Vickerman (1994 *Int J Parasitol 24*: 1317-1331) on the social organization and altruistic behavior (acting to increase another individual's output of offspring at a cost to one's own survival) of the parasite population. "A striking example of altruistic behavior is provide by the antigenic variation in the African trypanosomes. In the mammalian host the parasite population survives by sacrificing at regular intervals the majority of its members to host's immune response. Remission and relapse occur repeatedly and is evident by the fluctuating parasitaemia observed in humans. At each remission some member of the population lay down their lives so that their fellows can continue the line. This serial sacrifice prolongs the infection in the human and also increases the likelihood of uptake by the vector and transmission to another host".

It is not illogical a reflection on an altruistic behavior in *T. cruzi* populations with a similar strategy and its clinical and epidemiological consequences.