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TRYPANOSOMA CRUZI: MAJOR CLONES RATHER THAN PRINCIPAL ZYMODEMES

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The biological diversity of Trypanosoma cruzi has been explored with biochemical markers, such as isozymes (1) or kinetoplast DNA fragments (2). These studies, based on a phenetic interpretation of the variability observed, have led to descriptive concepts, such as zymodemes (a set of isolates that share the same isozyme profile) or schizodemes (a set of isolates that exhibit the same kDNA fragment pattern). On the other hand, a distinction has been introduced between principal and lesser zymodemes (3), the former ones exhibiting radical differences, the last ones being separated from each other by minor differences only.

We have analysed the isozyme variability (15 genetical loci) of the complex Trypanosoma cruzi and have interpretated our results in terms of population and evolutionary genetics, and of phylogenetic systematics. Our sample involves more than 500 stocks isolated from the whole ecogeographical range of the parasite, and from various hosts. In natural cycles of the parasite, a typical CLONAL STRUCTURE (4,5,6) has been evidenced, based on classical

sources of evidence in such studies, whatever be the organism considered (parthenogenetic animals or plants, bacteria, etc): fixed heterozygosity, alterne alleles in close sympatry (same host: Human patient, triatomine bug) with lack of the corresponding heterozygous recombinant, high statistical departures from Hardy-Weinberg expectations, strong linkage disequilibrium. All statistical results are constantly highly significant and corroborate the clonal structure.

It is worth comparing our results with the "clone concept" developed in bacteriology. Some bacterial cultures isolated from different sources, in different places, and at different times are so similar to each other that they must have derived essentially from a common ancestor by virtually complete asexual reproduction (7). Ochman and Selander (8) have evidenced such a basically clonal structure, with little or no chromosomal recombination, in natural populations of Escherichia coli, on the basis of isozyme analyses analogous to ours-- even though some recombination (parasexuality) can be experimentally induced in this bacterium. In the same way, even if genetic recombination could be experimentally obtained in T. cruzi, the clonal structure of its natural populations is apparent.

According to the clone concept, any two lines of genetical markers should yield similar inferred genetic relationships among

a given group of natural isolates. This has been verified in the case of E. coli between biotyping and isozyme analysis (9). Similarly, we have recently carried out a quantitative analysis of kDNA fragment patterns in a set of T. cruzi stocks previously characterized with 15 isozyme loci (10). The isozyme and kinetoplast DNA variabilities are highly correlated, which (a) favors the hypothesis of clonal structure in T. cruzi; (b) shows that the two sets of genetical markers (isozymes based on a sufficient range of loci and quantitative analysis of kDNA fragments) corroborate each other and provide valuable phylogenetic tools.

A third line of genetical markers (quantitative analysis of surface-polypeptide and -antigen variability: Brenière & Tibayrenc, in preparation) has also revealed significant correlation with isozyme variability, which corroborates the clonal structure in T. cruzi.

These results show that T. cruzi zymodemes and schizodemes are NATURAL CLONES (or families of closely related clones) of the parasite, as they can be individualized using the corresponding biochemical markers. Using a broader range of markers should result in distinguishing a higher number of clones for a given stock sample, which makes unwise to settle any numbering or labelling of the natural clones that would be definitive.

This clonal model may have obvious medical implications: the natural clones of the parasite represent discrete genetic entities or "agamospecies", which biological characteristics (including medically important ones) cannot be exchanged from one clone to another. These natural clones are genetically highly diversified and exhibit a continuum of genetic distances, which makes arbitrary their clustering into a few, well-defined principal groupings, or "principal zymodemes".

The distribution of the natural clones reveals a striking feature: also 43 different isozyme genotypes were identified, most of them appear as exceptional, since they were sampled only one time, or a few times. On the contrary, a limited number of them, which we call MAJOR CLONES, were repeatedly sampled in geographically distant places and various hosts. For instance, one of them was observed in Chile, Brazil, Venezuela, Colombia and Bolivia, in selvatic as well as in domestic cycles. Out of 43 isozyme genotypes, we have identified four ones which could represent such ubiquitous major clones. They represent more than 50% of a set of 121 stocks (5) collected over the whole ecogeographical range of the parasite. The major clones could represent "general purpose genotypes" (6), and their epidemiological and medical significance might be considerable. It is worth noting that the major clones already recorded by us do

not correspond to the principal zymodemes previously described in T. cruzi (3).

Work is in hand to complete the picture of genetical diversity of T. cruzi, and to explore the biological and medical importance of the already recorded major clones.

It is possible that the clonal model proposed here for Trypanosoma cruzi could be fruitfully extended to African trypanosomes: indeed although genetical recombination has been experimentally obtained in Trypanosoma brucei (11), statistical analyses similar to ours, carried out on natural populations of this parasite, have recently evidenced marked linkage disequilibria in trypanosomes isolated from tsetse flies as well as from Human patients (12). This result strongly suggests a clonal structure of the T. brucei natural populations involved in this analysis.

References:

1. Miles, M.A. et al. (1977): The identification by isozyme patterns of two distinct strain-groups of Trypanosoma cruzi, circulating independently in a rural area of Brazil. Trans. R. Soc. Trop. Med. Hyg., 71: 217-225.

2. Morel, C.E. et al. (1980): Strains and clones of Trypanosoma cruzi can be characterized by pattern of restriction endonuclease products of kinetoplast DNA. Proc. Nat. Acad. Sci. USA, 77: 6810-6814.

3. Miles, M.A. (1985): Isozyme characterization. Rev. Soc. Brasil. Med. Tropical, 18 (suplemento): 53-59.

4. Tibayrenc, M. et al. (1981): Arguments génétiques contre l'existence d'une sexualité actuelle chez Trypanosoma cruzi; implications taxinomiques. C. R. Acad. Sci. Paris, 293: 207-209.

5. Tibayrenc, M. et al. (1986): Natural populations of Trypanosoma cruzi, the agent of Chagas'disease, have a complex multiclonal structure. Proc. Nat. Acad. Sci. USA, 83: 115-119.

6. Tibayrenc, M. & Ayala, F.J. (1988): Isozyme variability in Trypanosoma cruzi, the agent of Chagas'disease: genetical, taxonomical and epidemiological significance. Evolution, 42: 277-292.

7. Ørskov, F. and Ørskov, I. (1983). Summary of a workshop on the clone concept in the epidemiology, taxonomy, and evolution of the Enterobacteriaceae and other bacteria. J. Infec. Dis., 148: 346-357.

8. Ochman, H. and Selander, R.K. (1984). Evidence for clonal population structure in Escherichia coli. Proc. Natl. Acad. Sci. USA, 81: 198-201.

9. Miller, R.D. and Hartl, D.L. (1986). Biotyping confirms a nearly clonal population structure in Escherichia coli. Evolution, 40: 1-12.

10. Tibayrenc, M. & Ayala, F.J. (1987): Forte corrélation entre classification isoenzymatique et variabilité de l'ADN kinétoplastique chez Trypanosoma cruzi. C. R. Acad. Sci. Paris, 304: 89-92.

11. Jenni, L.S. et al. (1986): Hybrid formation between African trypanosomes during cyclical transmission. Nature, 322: 173-175.

12. Cibulskis, R.E. (1988): Origins and organization of genetic diversity in natural populations of Trypanosoma brucei. Parasitology, 96: 303-322.