481

Trypanosoma cruzi THE AGENT OF CHAGAS'DISEASE, IT IS CLONAL AT MICROEVOLUTIONARY LEVEL?: EPIDEMIOLOGICAL IMPLICATIONS

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It has been proposed that *Trypanosoma cruzi* has been a clonal population structure and asexual reproduction, based on the deviation from Hardy-Weinberg equilibrium observed in natural populations. However, it is difficult to get a clear idea of the relative heterogeneities when we study several isolated of one only host.
Here, we have studied ninety four stocks of *Trypanosoma cruzi* isolated (from 1 to15 isolated per child) from children population in Cochabamba (Bolivia), All stocks were analysed by Multilocus Enzyme Electrophoresis (21 locus).
This study showed a genotypic microvariability among stocks pertaining to different clonal genotypes 20 to 39, according to the numbering by Tibayrenc and Ayala (1998). The within-patient microvariability was important (from 1 to 9 genotypes by patient). No statistical test was possible to use for the genetic analysis of the stocks on each patient. However, when performed on the whole sample of patients, significant linkage disequilibrium was revealed.

482

MOLECULAR CLONING OF A PUTATIVE TELOMERIC/SUBTELOMERIC SEQUENCE OF *Trypanosoma cruzi* (clone CL Brener)

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We have constructed four telomeric libraries in pUC 18 vector using genomic DNA fragments obtained by partial digestion with exonuclease Bal31 or mechanical shear. High molecular weight genomic DNA was partially digested with different concentrations of Bal31. After filling of the ends with DNA polymerase I (Klenow fragment), chromosomal DNA was ligated to pUC18 plasmid linearised by *Sma*I digestion. Ligated products were digested with *Eco*RI and circularised by ligation with T4 DNA ligase. Three telomeric libraries were generated with this procedure. Another telomeric library was constructed with genomic fragments cleaved by mechanical shear, repaired with DNA polymerase I (Klenow fragment) and inserted into the *Sma*I site of pUC18.
White *lac*$^-$ colonies were randomly chosen and the inserts amplified by PCR using primers derived from vector sequences. One recombinant clone (CL9) obtained by Bal31 digestion, about 1 kb long, was characterized. The BLASTn search analysis showed that the first 50 bp of CL9 shares 80% homology with the 3' UTR of gene TSA-1 (nt 3570-nt 3620) which encodes a *T. cruzi* trypomastigote stage-specific surface glycoprotein of 85 kDa. TSA-1 gene is a telomeric member of gp85 characterized by its inability to be ordinary cloned and Bal31 sensitivity (Peterson et al. EMBO J. 8: 3911, 1989). CL9 clone presents some other features that are characteristic for a sequence being telomeric. In Southern blot analysis, CL9 clone mainly hybridized with a 20 kb-fragment obtained by digestion with several restriction enzymes. Restriction genomic analysis revealed the existence of a universal restriction site, suggesting a chromosome end. Cl9 clone hybrized with the majority of *T. cruzi* chromosomal bands separated by pulsed field gel electrophoresis.
Three other Bal31 libraries were constructed using DNA from different *T. cruzi* strains: G, Tulahuen (Tu) and Y. We have sequenced one clone from each library: clone G-5 (390 bp), clone Tu-3 (350 bp) and clone Y-5 (343 bp). By BLASTn search analysis we have found that they share no homology with any GenBank sequence. These putative telomeric sequences present some interesting structural features: a) they share 55-65% homology among them, b) they carry several direct and inverted repeats and a consensus sequence $(C)_{1-4}(T)_{1-6}$ (C) repeated several times.

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