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Short Communication

Subspecific kDNA probes for major clones of *Trypanosoma cruzi*

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Trypanosoma cruzi, the agent of Chagas' disease, exhibits high genetic variability, as shown by zymodeme and schizodeme analysis (Miles et al., 1977, Morel et al., 1980). Extensive population genetics have demonstrated a basically clonal population structure of the natural population of *T. cruzi*, and have shown that the zymodemes and schizodemes can be equated to natural clones (or families of closely related clones) of the parasite (Tibayrenc et al., 1986; Tibayrenc and Ayala, 1988). Among the *T. cruzi* clones, a limited number are repeatedly sampled over large geographical areas and long periods of time. These widespread clones have been named 'major clones': their medical and epidemiological characteristics deserve to be investigated with utmost priority (Tibayrenc and Brenière, 1988). We present here results concerning DNA probes specific for two of the major clones. The polymerase chain reaction (PCR) was carried out in order to generate selectively important amounts of high variable regions (HVRm) from the kDNA minicircles of *T. cruzi*. Oligonucleotide sequences (20–23) were selected from high constant regions of the minicircle (Degrave et al., 1988; Gonzalez, 1986; Macina et al., 1986) in order to anneal sites flanking the variable region. An artificial restriction site was introduced close to the 3'-ends of each oligoprimer, allowing a fast and easy purification of HVRm (250 bp band) ready to use as probe.

We successfully amplified 250 bp major HVRm fragments from the total DNA of 26 genetically highly diversified *T. cruzi* stocks, isolated from various places and hosts (Tibayrenc and Ayala, 1988). The sensitivity of PCR was estimated by titration; the minimal amount of total DNA used as template was 10^{-4} fg. Moreover, lysed cells were tested by PCR, a positive amplification was obtained from as few as 1–5 cells (epimastigote or trypomastigote forms). We have screened HVRm probes from

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two different stocks representing each a major clone, namely SO 34, clone (or 'zymodeme') 20, stock SC43 cl1, clone (or 'zymodeme') 39 (the clone numbering used here refers to Tibayrenc and Ayala, 1988). These probes were hybridized with homologous total DNA, and the total DNA from 24 other stocks (see Fig. 1). Probe radiolabelling (alpha ^{32}P dCTP) and hybridization were carried out according to the commercial Rapid Hybridization System-Multiprime (Amersham®, U.K.). Non-radioactive labelling and hybridization conditions were carried out according to the commercial kit of Boehringer®, F.R.G. SO34 and SC43 cl1 HVRm probes specifically recognized their own stocks (data not shown) and all others pertaining to their own clone (or 'zymodeme') (respectively 3 and 5 stocks isolated from various places), and no other stocks pertaining to other clones (see Fig. 1).

Several authors have presented DNA probes specific to the whole taxon *T. cruzi*, used for parasite detection in host blood or in vector feces (Gonzalez et al., 1984; Creig et al., 1987; Sturm et al., 1989), or specific to 'strains', but without any clear genetical definition of these strains (Macina et al., 1987). PCR probes have been

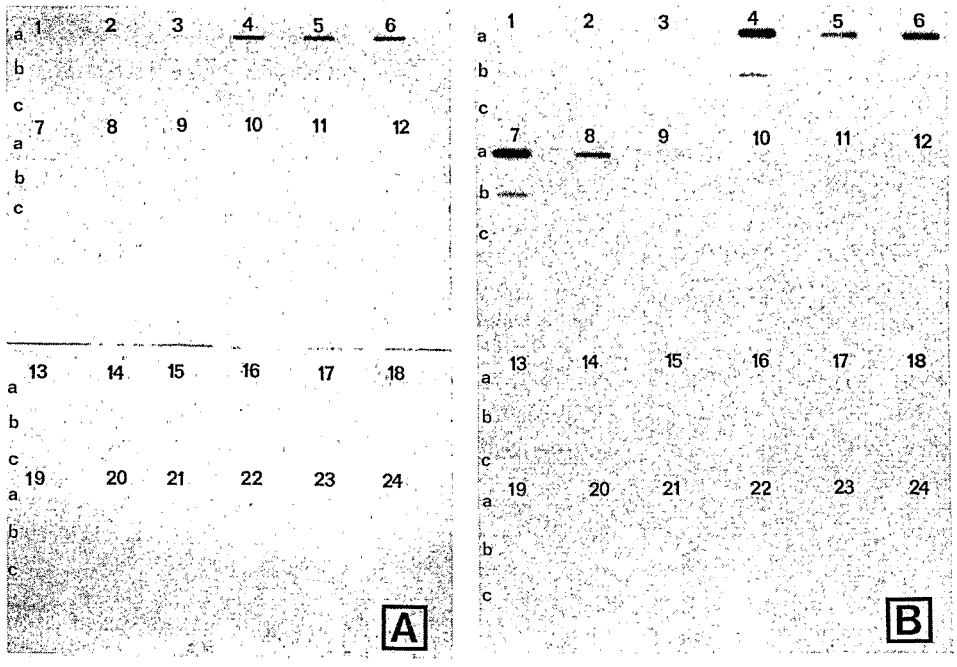

Fig. 1. Hybridization of two separate slot blot sets of total DNAs from 24 different *T. cruzi* stocks (representing 12 different natural clones) at three decreasing dilutions (a) 20 ng; (b) 2 ng; (c) 0.2 ng. (A) Non-radiolabelled SO34 HVRm probe (clone 20) was hybridized with stocks pertaining to: clone 17 (slot 1); clone 19 (slots, 2, 3, 7, 8); clone 20 (slots 4, 5, 6); clone related to 19 (slots 10, 11); clone 27 (Slot 12); clone 30 (slot 13); clone 32 (slot 14); clone 33 (slot 15); clone 35 (slot 16); clone 36 (slot 17); clone 39 (slots 18 to 23); clone 43 (slot 24). (B) Radiolabelled SC43 cl1 HVRm probe (clone 39) was hybridized with stocks pertaining to: clone 43 (slot 2); clone 36 (slot 1); clone 35 (slot 3); clone 39 (slots 4 to 8); clone 33 (slot 9); clone 32 (slot 10); clone 30 (slot 11); clone 27 (slot 12); clone related to 19 (slots 13, 14); clone 20 (slots 15, 16, 17); clone 19 (slots 21, 22, 23); clone 17 (slot 24). Clone numbering refers to Tibayrenc and Ayala (1988). The membranes were washed at the same stringency conditions (0.1 SSPE or 0.1 SSC and 0.1% SDS) and then autoradiographed on MP films (Amersham®, U.K.) for 20 h. Both probes hybridized specifically only with stocks pertaining to their own clone (or 'zymodeme'), 3 and 5 stocks respectively.

recently proposed as a sensitive diagnosis for Chagas' disease (Sturm et al., 1989; Moser et al., 1989). The present results involve PCR probes specific to fully characterized subdivisions of the taxon *T. cruzi*, the major clones, defined by a population genetics approach. It is worth noting that the success in obtaining probes specific for given zymodemes depends upon, and supports the hypothesis of clonal structure in *T. cruzi*. This success also suggests that the kinetoplast and nuclear genes are linked, a result already supported by the correlation between kDNA and isozyme variabilities (Tibayrenc and Ayala, 1988).

These probes (specially the non-radiolabelled ones) will make it possible to easily assess clinical and epidemiological studies of *T. cruzi* below the species level, on a rigorous genetical basis. Numerous important points about the behaviour of the major clones may be clarified by using these probes: their geographical patterns, their possible relationships with clinical forms of Chagas' disease, and the importance of mixed infection by several clones within the same host, already evidenced by isozyme analyses (Brenière et al., 1985; Tibayrenc et al., 1985).

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