Rapid identification of *Trypanosoma cruzi* isolates by 'dot-spot' hybridization

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The presence of isolate-specific *Trypanosoma cruzi* minicircles has been shown in the kinetoplast DNA of this parasite. This led to the rapid identification of isolates and clones of trypanosomes by means of 'dot-spot' hybridizations with molecularly cloned minicircle probes. Unexpectedly, whole kDNAs were also suitable as probes for this purpose, provided that filters were washed under stringent conditions. This was attributed to the presence of the above-mentioned isolate-specific minicircle sequences. The fact that parasites could be directly spotted onto nitrocellulose filters simplified the rapid routine screening of a large number of samples.

Trypanosoma cruzi *Kinetoplast DNA* *Minicircle* *Minicircle cloning* *Isolate-specific minicircle* *Isolate identification*

1. INTRODUCTION

The hemoflagellate parasite *Trypanosoma cruzi* (*T. cruzi*) is the agent of Chagas' disease. It is believed to group a number of heterogeneous trypanosomes as judged by the different biological and biochemical features observed among isolates [1–3]. Thus, a rapid identification method for *T. cruzi* isolates and clones would be a valuable tool not only for field studies but also for laboratory work. Among the most powerful methods used are isoenzyme analysis [4,5] and restriction enzyme patterns from the mitochondrially located kinetoplast DNA (kDNA) [3,6], both of which, however, are time-consuming.

The kDNA is a distinguishing feature of the order Kinetoplastida, which includes trypanosomes, leishmanias and crithidias. This kDNA is made up of two groups of catenated circles, namely a minor fraction of large circular transcribed DNAs (the maxicircles) and a major fraction of small molecules (the minicircles) whose function is unknown [7]. The latter are heterogeneous in sequence [8,9] and their rapid evolution allowed us to locate a minicircle only found in certain *T. cruzi* isolates [10]. This and other newly cloned molecules were used here to detect specifically parasite isolates in dot-spot hybridization experiments. Moreover, we showed that whole kDNA could also be used as specific probes provided that the filters were washed under stringent conditions. This result, rather unexpected as most minicircles from different *T. cruzi* isolates share homologies [9], allowed the direct use of whole kDNA as probe, parallel to the procedure in [11] for *Leishmania* species identification.

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Abbreviations: kDNA, kinetoplast DNA; SSC, 150 mM NaCl/15 mM sodium citrate; kb, kilobasepairs

2. MATERIALS AND METHODS

2.1. *Parasites*

Most of the *T. cruzi* isolates have been described

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in [10], and were used without cloning but for the CA-1 (no.70,71) and Miranda (no.78,83) isolates which were grown from a single parasite [12]. Trypanosomes were grown in liquid or biphasic medium as in [6,10].

2.2. *kDNA isolation*

Parasites were washed in 0.154 M NaCl and their kDNA purified by a quick isolation procedure [6].

2.3. *Dot-spot and filter hybridization*

Purified kDNA or parasites from each isolate were spotted onto nitrocellulose filters in a final volume of 5 μl. Denaturing was performed once on Whatman paper soaked in 0.5 M NaOH, after which neutralization was carried out twice on paper soaked in 1.5 M NaCl/0.5 M Tris (pH 7.4). Filters were then washed in 3 × SSC and dried for 2–3 h at 80°C. Prehybridization (2 h) and hybridization (12–18 h) were performed in the medium described in [13] with 'nick translated' DNA probes [14]. Filters were washed at 65°C in 0.1 × SSC/0.1% SDS. In some cases filters were reused after melting during 15 min in 0.1 M NaOH and washed twice in 3 × SSC. Transfer of digested kDNA electrophoresed in 2% agarose gel was done as in [15]. Hybridization and washing of the filters were performed under the conditions described for dot-spot hybridization.

2.4. *DNA probes*

Total kDNA or molecularly cloned minicircles were used as labeled probes. Recombinant clones pTck-12 and pTck-14 were isolated from *T. cruzi* Tul O kDNA and have been described in [9]. pTckAWP-2 and pTckAWP-3 were cloned in the *Hin*dIII site of pBR322 from AWP kDNA digested with the same restriction endonuclease.

3. RESULTS

Two concentrations of purified kDNA were spotted onto nitrocellulose filters and hybridized with 4 cloned minicircles, two from Tul O *T. cruzi* (pTck-14 and pTck-12) and the other two from AWP (pTckAWP-2 and pTckAWP-3). After washing the filters in 0.1 × SSC at 65°C, pTck-14 and pTckAWP-2 signals were clearly distinct for different isolates (fig.1). Thus, pTck-14 detected

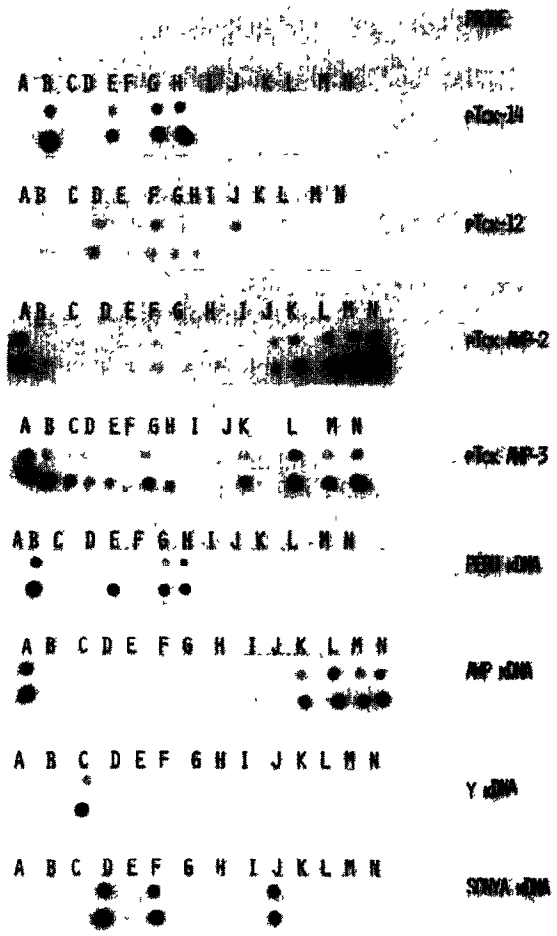

Fig.1. Dot-spot hybridization with different *T. cruzi* isolates. Two concentrations (0.08 and 0.4 μg, first and second row in each autoradiogram, respectively) of kDNA from the given *T. cruzi* isolates were spotted onto nitrocellulose filters and hybridized with either cloned minicircles (pTck) or whole kDNA. Filters were washed in 0.1 × SSC and exposed to Kodak X-Omat R film with intensifying screens. A, AWP; B, Peru; C, Ipsilon; D, Sonya; E, Tulahuen; F, TN; G, RA; H, UP; I, Mg; J, FN; K, CA-1 70; L, CA-1 71; M, Miranda 78; N, Miranda 83.

Tul O, Peru, RA and UP isolates while pTckAWP-2 hybridized with AWP and the 4 cloned parasites from CA-1 and Miranda. On the other hand, pTck-12 and pTckAWP-3 failed to provide reliable signal/background ratios.

Similar filters containing kDNA spots were probed with labeled kDNA (fig.1). Unexpectedly, they specifically detected certain *T. cruzi* isolates.

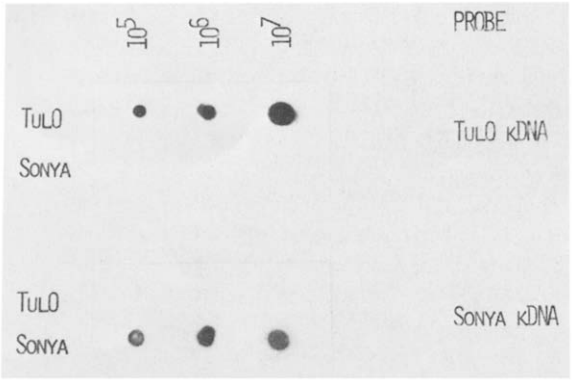

Fig.2. Dot-spot hybridization with whole parasites. Three amounts of parasites from Tulahuen Tul O and Sonya *T. cruzi* isolates were spotted onto nitrocellulose filters. Duplicate filters were denatured and neutralized as described in section 2 and hybridized with labeled kDNA from Tul O or Sonya isolates.

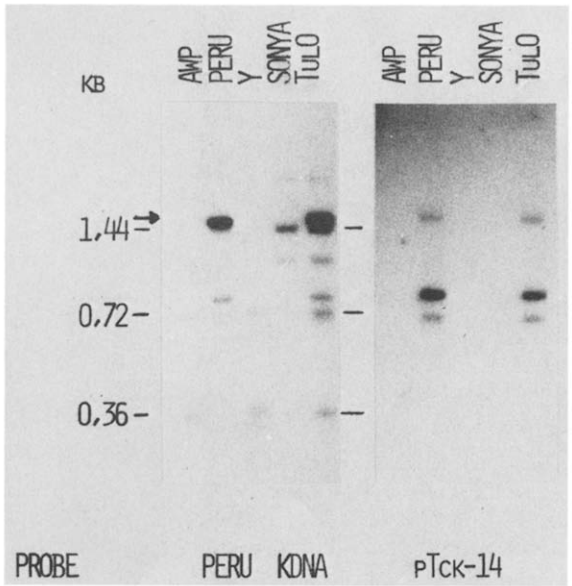

Fig.3. Location of isolate-specific minicircle sequences in kDNA restriction endonuclease digests. Purified kDNAs from the stated *T. cruzi* isolates were digested with *Hae*III, electrophoresed in duplicate in 2% agarose gels and blotted onto nitrocellulose filters. After hybridization with nick translated kDNA from Peru (A) or pTck-14 (B), the filters were washed in 0.1× SSC at 65°C and exposed as described in fig.1. *Hae*III-digested ϕX174, replicative form, was used as M_r marker.

Thus, Peru and AWP specifically identified the same isolates as did pTck-14 and pTckAWP-2, respectively. Y kDNA lighted up its own spot while Sonya hybridized with itself, TN and FN. Similar results were obtained when parasites, instead of purified kDNA, were spotted onto nitrocellulose filters and hybridized with labeled kDNA (fig.2).

We found previously that most cloned minicircles from *T. cruzi* Tul O hybridized with all stocks tested even under stringent conditions [9]. To unravel the unexpected specific hybridization achieved with whole kDNA as probe, the experiment was repeated with digested kDNAs from isolates that were either positive or negative with kDNA from Peru in dot-spot hybridizations (fig.3). Although several bands lighted up in all isolates, Peru and Tul O showed at least one strong hybridizing fragment not found in the other isolates (see arrow in fig.3). In addition, the latter hybridizing fragment was not a pTck-14-type minicircle, whose bands could also be visualized, as seen by comparison of panels A and B in fig.3. Consequently, it seemed that more than one isolate-specific minicircle was present in these kDNA networks.

4. DISCUSSION

We have demonstrated here that certain cloned minicircles as well as whole kDNA are sensitive probes to identify *T. cruzi* isolates and clones. The fact that intact parasites directly spotted onto nitrocellulose filters could be used makes this method simple and rapid for routine screening of a large number of samples.

It may be concluded that kDNA minicircle sequences as a whole have diverged widely enough to be used for the detection of *T. cruzi* isolates. However, when cloned minicircles were used as probes some of them proved more specific, while the less specific ones might explain the observed background in hybridizations performed with kDNA as probe. It is noteworthy that when kDNA sequences were conserved among isolates, some of the individual minicircles making up these networks were also preserved. In fact, these characteristic minicircle subpopulations may explain the specific reactions obtained with whole kDNA, as seen in fig.3. Thus, although AWP, CA-1 and Miranda depicted somewhat different

kDNA restriction endonuclease patterns, they all carried a homogeneous pTckAWP-2 minicircle subpopulation and were positive with this probe in the dot-spot experiments (fig.1 and unpublished). The latter fact demonstrated that kDNA restriction endonuclease analysis and kDNA hybridization may not always give identical results, and may actually supplement each other. On the one hand, kDNA restriction endonuclease patterns may detect minor variations even if the isolates under study are related to each other; on the other, kDNA hybridizations may be more useful to group related isolates even if minor sequence variations due to the rapid evolution of minicircles are present.

In [10] we mentioned that the CA-1 *T. cruzi* isolate used contained pTck-14-type minicircles. This is not the case with the CA-1 cloned trypanosomes used here. This difference may have arisen from accidental mix-up of trypanosomes as they were grown in different laboratories. However, we are confident that field studies, together with the fast dot-spot screening procedure, may enable kDNA sequence conservation to be correlated with the geographical distribution of *T. cruzi* isolates.

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