RESEARCH NOTE

Detection of *Trypanosoma cruzi and Leishmania* Using the Polymerase Chain Reaction

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Direct detection and identification of *Trypanosoma cruzi* and *Leishmania* species in humans bearing Chagas' disease or leishmaniasis has an important clinical and epidemiological relevance. Both infectious agents have an intriguing host-parasite relationship; in chronic patients suffering from American trypanosomiasis very few circulating parasites are found in the blood, and in the case of cutaneous lesions caused by dermotropic *Leishmania* species the same phenomenon is observed.

The mitochondrial DNA of these parasites represents about 20% of the total cell DNA and is organized in a disk-shaped network where thousands of small interlocked minicircles represent 95% of this structure. These molecules show features that make them almost ideal as molecular targets, since they are present in the kinetoplast in a high copy number (about 10,000) and contain a conserved region of at least 120 bp, that can be evidenced in every molecule and a variable region that can be a source of population and sub-population specific probes (L Simpson 1987 *Ann Rev Microbiol 41*: 363-382). Based on these aspects, several molecular probes were generated in order to identify the presence of minicircle molecules in clinical samples. Although molecular probes offer an important resolving potential in the detection and typing of organisms, in many cases they present insufficient sensitivity.

After a more detailed analysis, and with the advent of the polymerase chain reaction (PCR) technique, very sensitive approaches could be developed for detection and subsequent typing of these kinetoplastid protozoa. In this report, we describe the use of minicircle molecule amplification, leading to the accurate diagnosis of Chagas' disease and leishmaniasis.

Oligonucleotides derived from the *T. cruzi* conserved region DNA sequence have been used in PCR based assays (Fig. 1), in order to detect this parasite in human blood samples (N Sturm et al. 1989 *Mol Biochem Parasitol 33*:205-214). We now routinely collect human blood samples (5 or 10 ml) in 6M guanidine-HCl/0.2M EDTA in a 1:1 ratio (HA Avila et al. 1991 *Mol Biochem Parasitol 48*: 211-222). The kDNA network is decatenated by boiling, thereby spreading the minicircle targets throughout the solution (C Britto et al. 1993 *Mem Inst Oswaldo Cruz 88*: 171-172). After this step the DNA is purified from a small aliquot by phenol extraction and precipitation, or using solid phase DNA binding products, and is submitted to the PCR reaction, which is carried out at 55°C, 72°C and 94°C, for 30 seconds each step, during 30 cycles. The amplified products are detected by gel electrophoresis and hybridized with an internal oligonucleotide probe. In previous studies, such an assay yielded a very high sensitivity and specificity. Indeed, Avila et al. (1993 *J Clin Microbiol 31*: 2421-2426) showed in a recent field study involving about 120 chronic chagasic patients that the PCR assay can attain 100% sensitivity and specificity as compared to combined serology and clinical diagnosis. Other authors also obtained very promising results showing that PCR positivity is by far better than any other direct parasitological method.

For *Leishmania* detection, a set of oligonucleotides flanking the conserved region of *Leishmania* minicircles (MR Rodgers et al. 1990 *Exp Parasitol 71*: 267-275) was used in PCR experiments with different clinical samples obtained from human cutaneous lesions and biopsies from dogs. The amplified minicircle products generated through this PCR analysis correspond to a 120 bp fragment of the conserved region of these molecules, once the oligonucleotides anneal

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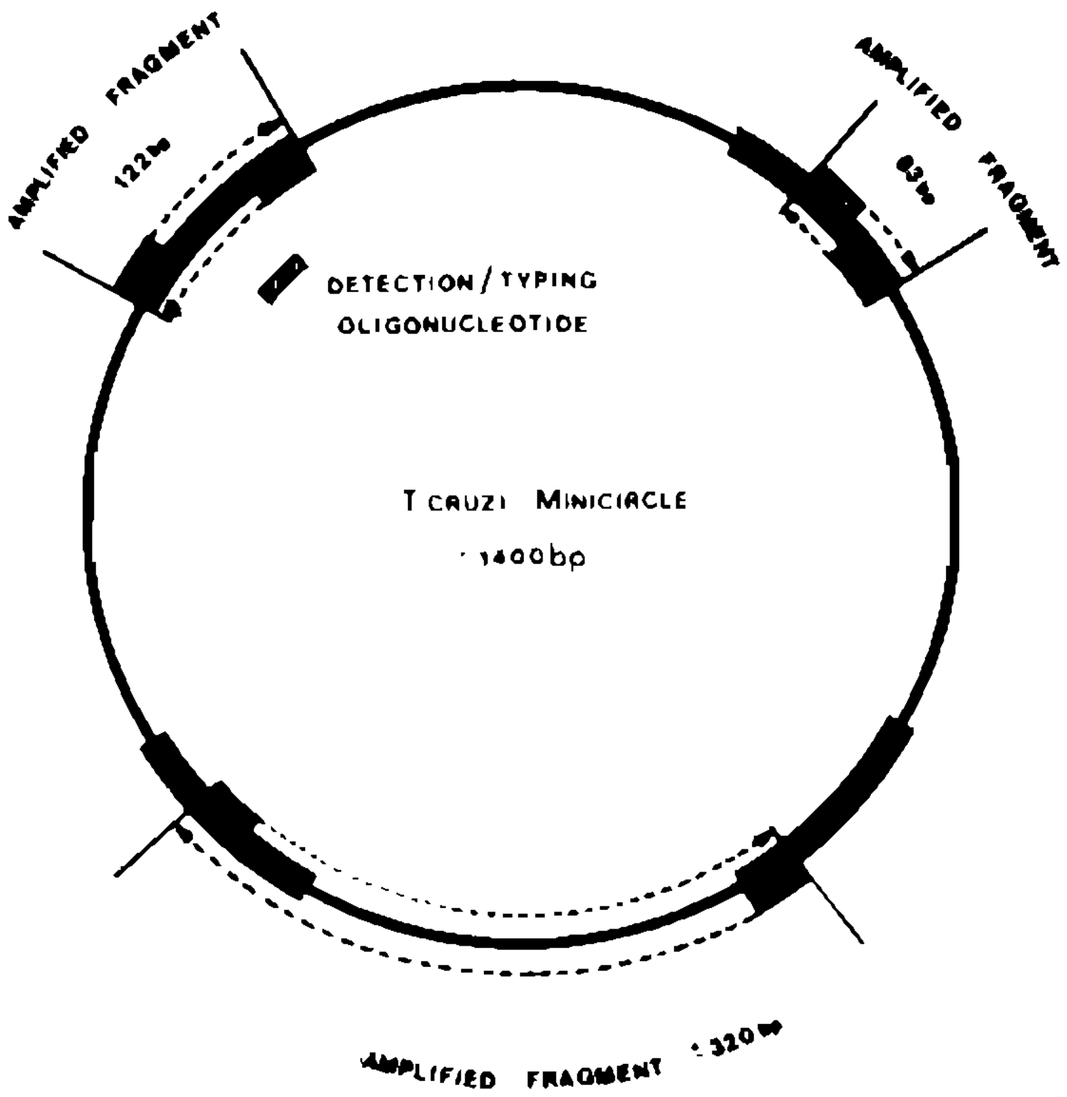

Fig. 1: *Trypanosoma cruzi* minicircle molecule showing the four conserved regions and the different PCR strategies for amplification of conserved and variable regions.

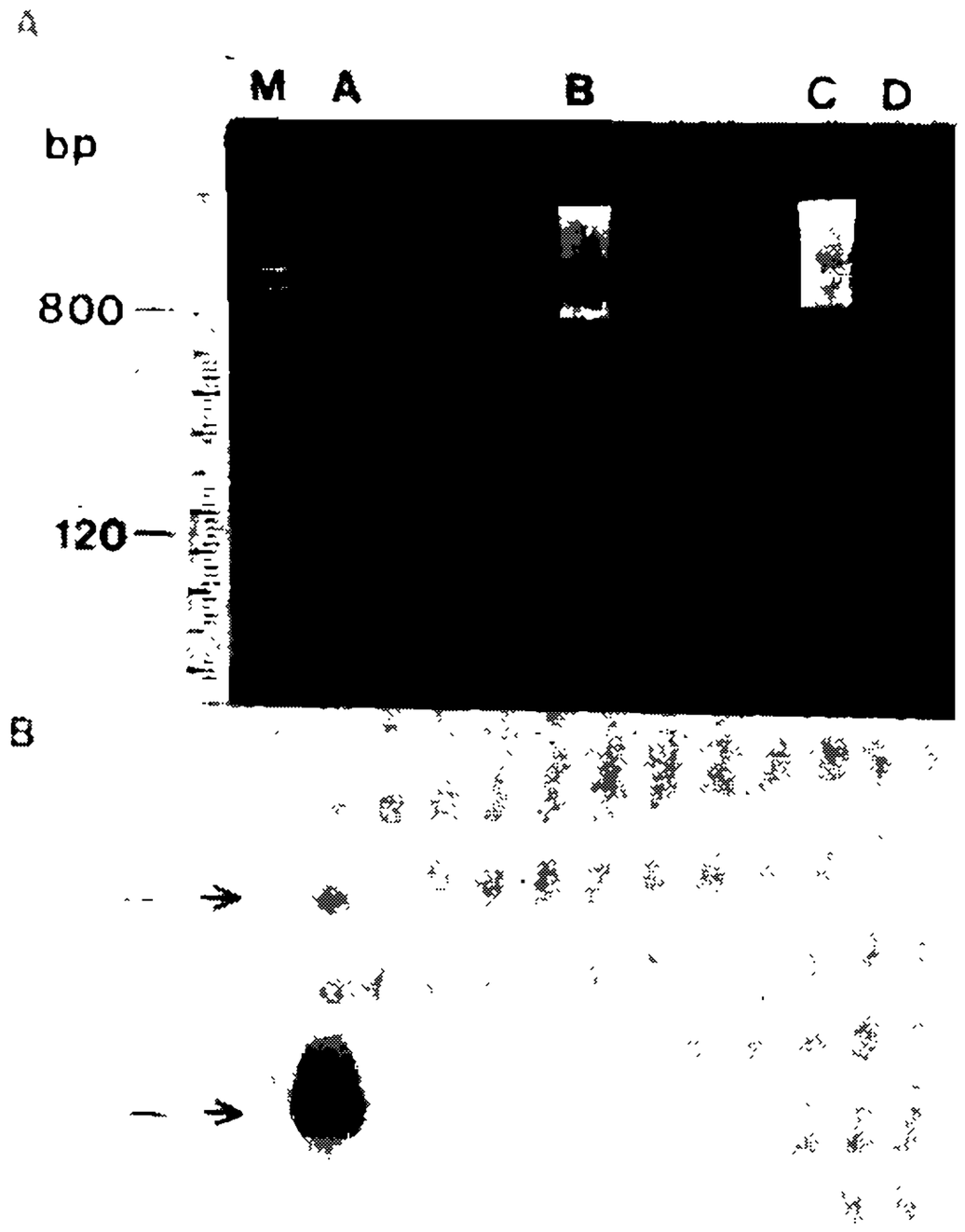

Fig. 2: A - Agarose gel electrophoresis of PCR amplified products from New World *Leishmania*. DNA was electrophoresed through a 2% gel and visualized by staining with ethidium bromide. Lane A, *L. braziliensis*; lane B, *L. amazonensis*; lane C, *L. chagasi*; lane D, negative control (no DNA added to the PCR reaction). In this experiment, strong amplification of whole minicircle molecule (800 bp) can also be evidenced. Molecular size marker (lane M) is ϕ X digested with Hae III. B - Southern blot of PCR amplified conserved regions from New World *Leishmania* hybridized with the sub-genus *Viannia* specific oligoprobe. The filter was hybridized in 4 X SSC at 37°C, washed in 0.5 X SSC at the same hybridization temperature, and exposed by autoradiography overnight with intensifying screen at -70°C.

to the putative origins of replication of the two strands. Through sequence analysis and multiple alignment of minicircle conserved regions from a variety of clinically important *Leishmania* species and from literature data, we designed modified sets of oligonucleotides which permit a very high sensitivity and can amplify all *Leishmania* species.

Consequently, it is useful to have different internal probes to type the amplified product. Our Laboratory can supply different molecular probes in order to characterize these products, once we have cloned minicircles from *L. panamensis*, *L. amazonensis* and *L. chagasi* (Leish-type kit) which, after hybridization experiments, can cluster the American leishmanias in three distinct groups: dermotropic (*Viannia*) sub-species; dermotropic *(Leishmania)* sub-species and the viscerotropic species *L. chagasi (W Degrave et al 1994 Mem Inst Oswaldo Cruz*, submitted). Furthermore, after sequencing and analyzing five different minicircle molecules of *L. (Viannia) braziliensis* (MHOM / Brazil / 75 / M2903), we were able to define 21 nucleotides of the conserved region that can be used as an oligoprobe (O Fernandes et al. 1991 *Mem Inst Oswaldo Cruz* 86 (suppl): 158) to type specifically amplified products from the sub-genus *Viannia* (Fig. 2).

Amplification products obtained with samples from human cutaneous lesions showed an expected size of 120 bp, which were derived from sub-genus *Viannia* minicircle molecules, once they hybridize with a cloned minicircle from *Leishmania panamensis*. In other set of experiments, biopsy samples from dogs, living in a major focus of kala-azar in Brazil, were also analyzed by PCR. The aim of this initial approach was to apply the PCR technique in the identification of putative animal reservoirs. Indeed, *L. chagasi* infection was detected in most of the analyzed samples, suggesting a widespread canine *Leishmania* infection in kala-azar foci. Our results demonstrated the potential of this methodology in the investigation of leishmaniasis foci.